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Aligning New Tuberculosis Drug Regimens and Drug Susceptibility Testing: A Needs Assessment and Roadmap for Action

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Abstract

New tuberculosis drug regimens are creating new priorities for drug susceptibility testing (DST) and surveillance. To minimise turnaround time, rapid DST will need to be prioritised, but developers of these assays will need better data about the molecular mechanisms of resistance. Efforts are underway to link mutations with drug resistance and to develop strain collections to enable assessment of new diagnostic assays. In resource-limited settings, DST might not be appropriate for all patients with tuberculosis. Surveillance data and modelling will help country stakeholders to design appropriate DST algorithms and to decide whether to change drug regimens. Finally, development of practical DST assays is needed so that, in countries where surveillance and modelling show that DST is advisable, these assays can be used to guide clinical decisions for individual patients. If combined judiciously during both development and implementation, new tuberculosis regimens and new DST assays have enormous potential to improve patient outcomes and reduce the burden of disease.

Keywords

Tuberculosis; drug susceptibility testing; diagnostics; drug regimens; modeling; surveillance; implementation; operational

Introduction

Health systems can be improved by two main pathways: the rethinking and reorganization of existing methods and technologies; and the introduction of new technologies. Over recent decades, national tuberculosis programs have used existing technologies more effectively and achieved significant impact.[1] But these responses are held back by the outdated and inadequate tools used to fight the epidemic: a vaccine with limited efficacy; a drug regimen that is long and that places significant demands on both patients and healthcare systems; and a diagnostic technique (smear microscopy) that only detects half of all cases and does not assess the drug resistance of the infecting *Mycobacterium tuberculosis* strain.[2]

As efforts accelerate to improve upon these tools, it is important to consider how they will work together in a health systems context. For example, failure to detect resistance can lead to inadequate treatments that are more likely to fail, while generating further resistance. Therefore, new tuberculosis regimens[3] cannot be introduced without developing drug susceptibility testing (DST) assays suited to the new regimens. This requires analysis of how new drugs and new diagnostics and DST should be used – in different epidemiological contexts – to monitor patterns of emerging drug resistance and to direct patients towards

appropriate therapy. This alignment of new tuberculosis regimens and new tuberculosis DST is the topic of this article.

The primary backbone for tuberculosis treatment has remained unchanged for decades; thus, susceptibility tests for additional agents have not received much attention.[4] But regimens with new tuberculosis drugs will change global priorities for DST and drug resistance surveillance. It will be particularly important to test for resistance against drugs in new first line regimens, especially since existing tuberculosis drugs are easily available in the private sector – in large volumes, and with little or no regulation – in many high tuberculosis burden countries.[5]

Through the Tuberculosis Diagnostics Research Forum, multiple partners are working to ensure that the necessary drug susceptibility tests are developed in time for co-implementation with new tuberculosis drug regimens. The aim is to develop a framework for designing DST for new regimens. As an immediate goal, such DST should meet at least the same performance criteria as DST for existing first-line therapy. The ultimate goal is to have sufficient information – including prevalence of existing resistance – so that all tuberculosis patients can be treated with a high level of confidence that their regimen will be safe and effective.

In reaching these goals there are multiple needs (Panel 1). Translational science is needed to provide the basis for molecular diagnostics development. Surveillance data and modeling are needed for designing DST protocols and for regimen change decision making. And, in countries where the surveillance and modeling indicate that this is necessary, DST assays are needed for clinical decision making for individual patients. In this review, we outline in more detail the actions required in each of these areas in order to maximize the impact of introducing new technologies in tuberculosis control.

Search strategy and selection criteria

This article draws on material from a meeting of the Tuberculosis Diagnostics Research Forum sponsored by the Bill & Melinda Gates Foundation and the US National Institutes of Health and held on October 1–2, 2012 in Arlington, Virginia. In addition, references for this review were identified through searches of PubMed with a focus primarily on articles published during the past 5 years. Search terms included, but were not restricted to, “tuberculosis”, “drug susceptibility testing”, “drugs”, “diagnostics”, “drug resistance”, “surveillance”, and “point-of-care testing”. There were no language restrictions. Additional information came from our personal collections of peer-reviewed papers, from the reference lists of identified papers, and from reviewers.

Tuberculosis regimens: past, current and future

First line tuberculosis treatment has gradually evolved from an initial monotherapy with streptomycin, to multidrug regimens of up to 24 months or more, and finally to the “short course” regimen currently used in most high burden countries.[6] The latter is a 6 month regimen denoted as 2HRZE/4HR: a 2 month intensive phase of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) followed by a four month continuation phase of

HR. This regimen has been a global standard for first-line tuberculosis treatment for decades.

The duration of the 6 month regimen continues to put significant demands on health care systems and patients.[7, 8] Meanwhile, second line tuberculosis treatment, required for those with multidrug-resistant TB (MDR-TB; defined by resistance to both isoniazid and rifampicin), is based only on observational studies and expert opinion.[9] These multi-drug regimens of 18–24 months are toxic, expensive, and of limited efficacy.[10] The inadequacy of these regimens, which has become more evident as greater numbers of people are diagnosed with MDR-TB, has led to increasing efforts to find and develop new tuberculosis drug regimens that would shorten first-line treatment, avoid the current drug-drug interactions with antiretroviral therapy, and improve second-line treatment.[3, 11]

Two phase III trials that aim to shorten first-line tuberculosis treatment have concluded patient enrollment and treatment. The OFLOTUB trial substituted the fluoroquinolone gatifloxacin for ethambutol in a 4 month treatment, although gatifloxacin subsequently lost regulatory approval in many countries based on adverse events. The REMoxTB trial substituted the fluoroquinolone moxifloxacin (M) for either isoniazid or ethambutol in two experimental, four month regimens (2HRZM/2HRM and 2MRZE/2MR). Results from REMoxTB are expected in late 2013; if positive, regulatory approval would be sought in 2014 and in-country launch may start as early as 2015.

Next-generation, first-line regimens are likely to include a number of novel drugs.[12] Clinically, the most advanced regimen[13, 14] in this category is PaMZ, a combination of the novel nitroimidazo-oxazine PA-824, moxifloxacin, and pyrazinamide. This regimen has the potential not only for treatment shortening for first-line treatment, but also for treatment of some proportion of patients who are currently classified as being in need of second-line treatment (i.e., patients with MDR-TB).[15]

Finally, there are several individual tuberculosis drug candidates that are in clinical development but for which optimized regimens have not yet been defined. Sutezolid (PNU-100480; Pfizer), an analogue of linezolid, is in Phase IIa trials. More advanced are two novel drugs that have been submitted for regulatory approval for treatment of MDR-TB, based on Phase IIb data. Bedaquiline (a diarylquinoline formerly known as TMC207; Janssen Pharmaceuticals)[16] and delamanid (a nitro-dihydro-imidazooxazole formerly known as OPC-67683; Otsuka Pharmaceuticals)[17] are supported by data showing faster reductions of bacterial burden when the novel drug was added, for 6 months, to an optimized background regimen for MDR-TB. Bedaquiline was granted marketing approval by the US Food and Drug Administration on December 31, 2012. However, the extent to which these drugs can shorten and simplify MDR-TB treatment will only be known after additional, multi-year Phase III trials.

Tuberculosis diagnostics and drug-susceptibility testing: past and current practice

For decades, tuberculosis diagnosis in high burden countries has relied almost entirely on smear microscopy, which is inexpensive but detects only half of all tuberculosis cases.[10] In addition, smear microscopy does not yield any information on drug resistance, so most patients are put directly onto a standardized first-line regimen without any knowledge of DST. However, the increasing awareness of MDR-TB[18] has drawn greater attention to the need for DST.

Recent developments in tuberculosis DST have progressed from centralized to more decentralized systems, with the biggest focus being rifampicin DST in order to diagnose MDR-TB. Development and field testing have led to WHO recommendations of automated liquid culture systems (in 2007), line probe assays (in 2008) and the Xpert® MTB/RIF test (in 2010; Cepheid, Sunnyvale, CA, USA). These systems offer benefits such as reduced time to detection of resistance (from effectively 106 days with conventional DST to 20 days for line-probe assay and <1 day for Xpert)[19] thus allowing for more rapid initiation of MDR-TB treatment.[20–22] They can be implemented, respectively, at the level of national and regional reference laboratories (for liquid culture and line probe assays) and sub-district laboratories (for Xpert, an automated, cartridge-based, real-time PCR).

Initially, phenotypic, culture-based methods were the primary method for conducting tuberculosis DST. For certain MDR-TB drugs even this phenotypic DST is not well established, and will require further research, as there are currently insufficient data to determine clinically relevant critical concentrations.[23] There are other phenotypic diagnostics such as the microscopic observation drug-susceptibility (MODS) assay and the nitrate reductase assay (NRA) that may be an interim solution for resource-constrained settings.[24] However, truly rapid testing requires a molecular approach, which is therefore the primary focus for this review.

Line probe assays, though molecular, also present challenges. As with liquid culture, they require laboratory infrastructure that is not available at the periphery, so they are not practical for routine testing of all tuberculosis suspects or patients in most high burden countries.[25] Such a step would require the establishment of a massive sputum sample referral and transport system that typically does not exist. Instead, cultures and line probe assays are used largely for patients at high risk of resistance (e.g., persistence of symptoms or failure to convert smear and culture results).

The Xpert test, however, opens up great possibilities due to its potential for use at the district or sub-district level. It also brings dual benefits: in addition to detecting rifampicin resistance, it detects far more tuberculosis patients than does smear microscopy, particularly in regions with a high number of people coinfected with HIV and tuberculosis.[19] As a result, Xpert has been adopted rapidly in South Africa, where it is used as the first diagnostic for all individuals with suspected tuberculosis. In other countries, such as Kenya, Xpert is used for all HIV-infected individuals with suspected tuberculosis. Other low resource countries, however, still struggle with the cost,[26] electricity, and maintenance requirements of Xpert.[27]

Although the price of the Xpert technology has been recently reduced to under $10 per cartridge, this negotiated price is not available to the large number[5] of TB patients in the private health sector in certain high burden countries.[28]

The ongoing roll-out of Xpert illustrates issues that will likely be applicable to DST development for new tuberculosis regimens. One significant issue is positive predictive value.[29,30] Even with a pooled sensitivity for rifampicin resistance of 94% and pooled specificity of 98%,[31] the latest iteration of Xpert has a positive predictive value (PPV) for MDR-TB of only ~50% or ~67% when rifampicin resistance prevalence is 1% or 2%, respectively.[29] Such resistance values are typical among new tuberculosis cases, and the low PPV results in a significant number of false positives and a significant demand for confirmatory DST.[32] (Of note, however, even culture is not a 100% accurate "gold standard", so the true specificity of the Xpert for rifampicin resistance may be higher than the initially reported 98%.) In many countries with lower HIV or MDR-TB prevalence, the issues of PPV and costs have limited the adoption of Xpert.

Future needs: bringing new tuberculosis regimens and new tuberculosis diagnostics together

Selecting drugs to test and ways to test them

Which of the new drugs are the most important targets for future DST? Typically DST would be prioritized to drugs for which resistance has one or more of three consequences: it seriously affects treatment efficacy; increases the risk of resistance amplification; or strongly predicts resistance to other drugs (i.e., acts as a triage assay). In the current regimen, rifampicin DST has been prioritized in order to diagnose MDR-TB.[33] Recent evidence suggests isoniazid DST should also be of interest: a significant pool of patients harboring isoniazid-resistant, rifampicin-susceptible strains exists, and patients with such strains have reduced treatment success.[34, 35] For implementation of the REMoxTB regimens (2HRZM/2HRM and 2MRZE/2MR), DST to rifampicin and moxifloxacin will be of interest, especially in countries that currently do DST for rifampicin. For PaMZ, a rapid test for moxifloxacin and pyrazinamide would likely be the first priority, as clinically significant resistance to PA-824 is not yet known to exist. Development of DST for PA-824 and other new drugs would be prioritized – initially for use in surveillance – as resistance to them develops and their use becomes more widespread.

But the needs do not stop at a description of which drugs to test. To be rapid, a DST assay will likely need to be molecular. Therefore, information about resistance mutations – and the correlation of those mutations with clinical outcomes – is needed to form the basis for such a test.

The Xpert achievement of 94% sensitivity for detecting rifampicin resistance is only possible because of two facts: almost every mutation contributing to rifampicin resistance is not only known, but present in a short, defined DNA region. For fluoroquinolones, however, incomplete knowledge of all contributing resistance mutations outside the QRDR region of *gyrA* and *gyrB* means that sensitivity with such molecular methods would, based on current knowledge, be limited to ~85%.[24, 36] As occurred recently for a line probe assay for second

line drugs, insufficient sensitivity can result in recommendation for use as a rule-in (triage) test only.[10, 32] Sensitivity might be further enhanced by incorporating additional, low abundance mutations but doing so may reduce specificity to an unacceptable level; for example, if specificity for each of five independent mutations is 98%, the overall specificity of a test including all five mutations would be 0.98^5 or 90%. Other major issues, for fluoroquinolones and other drugs, are the possibility of multigenic resistance, and the limit of detecting the already known mutations from a mixed population of bacilli.[37]

DST for pyrazinamide poses even more challenges. Pyrazinamide activation requires pH levels that are difficult to maintain in culture media, so phenotypic DST for pyrazinamide is inconsistent, and analysis of the sequence of a single resistance gene (*pncA*) has been proposed as an alternative.[38] The mutations are spread along the entire length of the *pncA* gene, however, necessitating analysis of a fragment of ~700 base pairs. This has led to the concept of testing for the presence of a wild-type gene (rather than testing for the presence of a specific mutation), as a way of ruling out resistance. Depending on how much faith is put in the phenotypic testing, only ~90% of pyrazinamide resistance may be explained by such *pncA* mutations.[38] Conversely, silent mutations, which do not confer resistance, would likely prevent hybridization and thus yield false positives. These silent mutations, although rare,[39] need to be better characterized by standardized and validated culture-based PZA resistance assays and incorporated into a molecular testing algorithm.

Given these limitations, one priority in translational science is to correlate gene mutations and the amount of drug needed to inhibit bacterial growth.[40, 41] A second priority is to develop strain collections (preferably sequenced[42, 43]) that would facilitate the evaluation of new diagnostic assays and the development of genomic databases for predicting drug susceptibility phenotypes. For new drugs, isolates that develop resistance in vitro should be stored for later evaluation, but their clinical significance will remain unclear until resistance is observed in clinical use. Compound availability for such clinical evaluation and data on critical breakpoints are likely to emerge only after regulatory approval of new tuberculosis drugs. Post-marketing studies will be important to identify treatment failures and to determine resistance mechanisms.

Surveillance: An early warning system and a basis for decision making

Once translational science has provided a means to detect resistance, the next task is to determine existing or emerging resistance levels via surveillance. Data for 2011 on global drug resistance collected through WHO's Global Project on Anti-TB Drug Resistance Surveillance is available from 135 countries out of 194 member states, of which only 63 countries have continuous surveillance systems using DST.[10] In general, surveillance is limited to activities that align with today's treatment priorities. Most countries assess resistance in new and retreatment patients to isoniazid, rifampicin and ethambutol (pyrazinamide is often excluded, due to the methodological challenges noted above). Resistance to fluoroquinolones is assessed only among MDR-TB patients, as this is the context in which these drugs are currently used in treatment regimens recommended by the World Health Organization and the International Union Against Tuberculosis and Lung

Diseases, although significant fluoroquinolone usage is believed to occur in first-line tuberculosis treatment in the private sector of some countries.[5]

Such data are insufficient to assess development and implementation priorities for new tuberculosis regimens and diagnostics. The key information gap for the REMoxTB regimens is fluoroquinolone resistance among new patients. Although existing data suggest that such resistance is very low in most[44–46] but not all[47, 48] countries, the absence of such data for most high burden countries makes it difficult for a country to assess the cost effectiveness of the new regimen (i.e., whether to implement) or the DST algorithm that would make most sense (i.e., how to implement; see below). And, for PaMZ, there is an additional gap of pyrazinamide resistance rates among both new and MDR-TB patients. For both moxifloxacin and pyrazinamide resistance, limited data are available from clinical trials, but nationally representative data are sorely needed. As new drugs with new mechanisms of action are adopted, surveillance will also be needed to monitor for the development of resistance to bedaquiline, delamanid, and others.

For surveillance data to be meaningful, the data must be representative of either a national or sub-national population, must be collected using quality-assured assays, and must distinguish between rates in new cases and retreatment cases. Ideally, DST surveys should be linked with patient care (although this would require methods with high quality-assurance) and treatment outcomes and would make use of new, high throughput molecular methods that would be much faster than the current growth based assays. For example, with a sufficient foundation of mutation data,[40] sequence-based assays can provide rapid and accurate information and, for many drugs, good correlation with DST obtained using liquid culture.[43]

DNA sequencing – as a centralized procedure – is more practical for surveillance than for patient care. But even for surveillance there must be attention paid to the development of fast and safe specimen preparation and transport that maintains stability of the DNA in the specimen. Templates, primers, barcodes, and standardized electronic reporting would have to be developed. Such systems should improve in accuracy as mutations with unknown association are collected and analyzed; while this knowledge is being accumulated, however, parallel implementation of phenotypic and molecular assays may be needed.

Working with a country with an ongoing drug resistance survey could provide an opportunity to pilot the technology and develop the systems described above. Such a study could provide the proof of principle and the data to validate such a system.

Modeling of alternative DST strategies

Drugs and diagnostics are implemented as individual elements of a larger, more complex tuberculosis management landscape. In the public health approach, all incoming patients are subdivided into a limited number of treatment pathways. Central to this landscape are diagnostic algorithms, which consist of different permutations of which drugs to test for, the level of the health care system at which the testing is done, the selection of the patient population eligible for testing, and decisions about single-step or multiple-step testing. At the end is a treatment decision. New regimens introduce multiple new variables to the

decision of which algorithms are most effective, and data to inform this decision will necessarily be limited at the time any new regimen is introduced.

Mathematical models can be useful tools for decision-making in such instances where direct data are limited.[49] Such models use existing data to simulate simplified tuberculosis epidemics that behave according to our best current knowledge. These models can then be used to project the medium-term incidence and prevalence of drug-resistant tuberculosis at the population level under various assumptions about the deployment of novel regimens and corresponding DST.

For example, one priority question is where DST should be placed in treatment algorithms for various epidemiological and economic contexts. Clearly, the ideal algorithm (from a perspective of limiting drug resistance) is to deploy DST for all tuberculosis suspects or all individuals with confirmed tuberculosis, with confirmatory testing of preliminary positives. Preliminary modeling has suggested that this type of “test early” strategy for isoniazid and rifampicin may be cost effective in areas with an underlying MDR-TB prevalence as low as 2.1%.[50] However, this is only feasible where good DST exists for a given regimen, resources are sufficient to deploy such DST widely, and where use of DST will not greatly delay the initiation of treatment. Most high-burden settings therefore cannot consider such algorithms at this time.

A history of prior treatment is a strong independent risk factor for resistance, so DST can be directed at these subpopulations. But when should DST be implemented more broadly? For large public health programs in lower-income high burden countries, it may make sense to implement DST only when the prevalence of resistance to a given drug rises above a certain threshold. Below this level, the implementation challenges and issue of false positives outweigh the risks from undetected resistance. Above this level, action is required to prevent poorer treatment outcomes, resistance amplification, and increased transmission. But in general the point at which this threshold should be set under different epidemiological and economic conditions remains unclear – especially when MDR-TB “hot spots” are present within countries that otherwise have low overall prevalence.[51] The answer will also vary depending on whether the remaining drugs in the regimen will still protect the person from resistance generation and disease progression. Modeling can provide input to the assessment of which thresholds make sense in terms of public health benefit, cost, and cost-effectiveness.

To support this assessment, two central questions have arisen in discussions of modeling DST in the context of novel tuberculosis regimens. First, what would different DST assays – with different speed, accuracy, price, and technical specifications (which drugs, how many mutations) – achieve in terms of population-level impact and cost-effectiveness, and what are the tradeoffs between these various specifications? Second, what is the population-level impact and cost-effectiveness of different DST algorithms (e.g., DST for all, DST for retreatment and failure cases only, or use of novel regimens without DST), as a function of baseline drug resistance and rate of emerging resistance?

The decision of how to deploy DST is a multifaceted one that must consider projected epidemiological impacts, budgetary constraints, feasibility concerns, and political realities. Mathematical models can assist with the first of these (projections of potential impact), and thereby serve as an important tool for decision-makers. However, these models are limited by the quality of data that inform certain assumptions; in particular, data remain sparse on existing levels of drug resistance in many high-burden areas and the rate at which resistance to second-line agents (e.g., fluoroquinolones) might emerge under pressure from new regimens. Thus, even when limited to the impact issue, mathematical models cannot validate which assumptions about emergence of drug resistance are the correct ones. However, they can project epidemiological outcomes under "best-guess" assumptions of these data points, describe the range of our current uncertainty, highlight the data for which surveillance is most critical as novel regimens are deployed, and provide preliminary guidance under our best current knowledge while those data are collected.

The development of new DST tools

The information about resistance rates (from surveillance) and algorithm choice (from modeling) can directly inform the final question: what new DST assays need to be developed? The target product profiles (TPPs) will vary depending on the intended use (individual treatment decisions versus surveillance), the epidemiology (detecting low versus high resistance), the health system context (where it is positioned in possible algorithms), and whether the technology will be used in centralized versus decentralized settings.

Example TPPs and DST methods have been described elsewhere.[52, 53] Beyond the target drug(s), these TPPs should address multiple issues: what is meant by "rapid"; what level of sensitivity and specificity a DST assay needs in order to be practical and implementable; what other diseases should be able to use the same DST platform technology; and what level of complexity, containment and cost are required.

But two related issues stand out. First, should DST be bundled into case-detection assays (as with Xpert), or should it be a reflex test that is done only after tuberculosis is diagnosed? The latter approach requires more patient samples (and potentially more patient visits, loss to follow-up, and delays in treatment initiation) but can greatly reduce costs if the DST functionality adds significantly to the cost of the case-detection tool.

Second, the new DST assays could be developed for deployment at either centralized labs or the more peripheral levels of the healthcare system (Table 1). There is no shortage of emerging technologies for DST – including microarrays; next-generation sequencing; line probe assays; molecular beacons; high-resolution melt curve analysis; lights on/lights off technology; cyclic catcher-melt temperature analysis; phenotypic color tests; pyrazinamidase assays; and combination phage and molecular assays – and some of these technologies can be readily adapted to increase the number of mutations detected. But few are suited to peripheral use.

Therefore, investment will be needed either to develop cost-effective and robust DST methods for peripheral laboratories, or to create rapid, reliable sample transport systems to underpin centralized DST (along with mobile-health and patient-incentive solutions to

reduce delays and dropouts). Deployment of testing at point-of-treatment can bring obvious advantages such as reduced delay and dropout, but can add significantly to the overall cost of testing due to the larger number of instruments needed and the lower volumes of testing per site in the periphery.[54]

Many countries diagnose drug-sensitive tuberculosis at the primary care and peripheral levels of the health system, but initiate treatment at the sub-district level. Therefore, a compromise may be to have a new, sensitive case detection assay as a true point of care assay, followed by DST administered as a reflex assay at sub-district level at the time of treatment initiation.

If non-centralized DST remains the strategy, simplicity should be a major goal.[55] Simplified smear microscopy algorithms provide an interesting example of how it is sometimes worth sacrificing some up-front performance (in this case, sensitivity) in return for a protocol that is simpler for the patient (with lower travel costs) and that therefore has less drop-out and better overall effectiveness.[56, 57] Modeling has already resulted in a similar conclusion for new diagnostics. Improved assay sensitivity yields some epidemiological gains, but the greater population impact comes from a focus on test specifications that allow peripheral use and fast turn-around times – thus reducing patient delays and default.[58,59]

One option for a peripheral test is to focus on excluding all those who are likely to be resistant; high sensitivity becomes the goal and specificity becomes less important. A test with lower specificity can be acceptable if the prevalence of resistance is high, or there is an effective and safe alternative regimen (e.g., 2HRZE/4RH for PaMZ), or for a triage test in a triaging strategy. This might be the case, for example, with a molecular assay to screen for the wild type *pncA* gene as a correlate for pyrazinamide susceptibility, rather than trying to capture the numerous different *pncA* mutations that can lead to pyrazinamide resistance. Another option is to continue – even with new regimens – with a focus on rifampicin resistance screening as a first step. Preliminary evidence suggests that there is much higher resistance to pyrazinamide and fluoroquinolones among rifampicin-resistant strains, so DST for rifampicin may be a useful triage test even if the first line regimen does not itself contain rifampicin (e.g., PaMZ), as the subsequent pyrazinamide and fluoroquinolone DST will be restricted to smaller populations with a higher prevalence of resistance.

Beyond all of these theoretical issues, the field needs the involvement of diagnostics developers who are aligned to what is needed in the field and willing to take a product all the way through field testing to commercialization. The perception of limited commercial opportunity remains a significant barrier to the development of the necessary tools, and supportive financing will likely still be needed. In addition to the TPP issues listed above, diagnostics developers are interested in potential market size and the practical steps required for test development, validation, regulation and policy (Panel 2).[60]

Developers targeting surveillance have a particularly small market, although the barrier to entry is much lower as these high throughput, centralized machines can be built presuming a higher level of user skill and applications beyond tuberculosis. For developers interested in peripheral DST for patient care, the demands in terms of assay simplification and robustness

increase greatly, and market size is very dependent on the resistance thresholds for testing. Test developers may therefore be more interested in a product that combines tuberculosis detection and DST as this will ensure a larger market than a DST-only product.

Private sector procurement remains a major strategic gap. If new DST assays are highly priced, few private practitioners will use them – and DST will be missing from the sector that is most likely to adopt new drugs quickly and in the context of variable regimens. To solve this issue, a mechanism is needed to ensure that private laboratories pass along any savings from assays purchased at concessionary prices.

Although demonstration projects for diagnostics require considerable investment, assay development using existing platforms can be relatively cheap. But even to make these investments, diagnostic companies need a prediction of user needs (where the “user” is often national tuberculosis programs) and market demand. Defining a clear set of specifications for the desired DST – and the likely demand for such DST – is the next major point of collaboration for drug and diagnostic developers.

Conclusion

The prospect of new tuberculosis regimens is bringing excitement to a field that has had to rely on a single, lengthy treatment option for decades. However, there are threats to this promise. Rapid development of resistance could occur if new drugs are added to failing regimens, or if there is widespread use of combination regimens in populations that have substantial existing resistance to some of the drugs in those combinations. In some cases this may result in monotherapy with a single new drug, increasing the chance of developing resistance and severely limiting the antimicrobial arsenal even further.

There are, however, multiple opportunities to mitigate these risks. Assays to detect resistance can be developed either before re-purposed drugs come to market or early in the implementation of new drugs. Surveillance DST can identify areas where some regimens might be compromised by high levels of background resistance and treatment decisions can, in some settings, be individualized by pretreatment rapid DST. Modeling will help to balance costs, outcomes and feasibility to predict implementation approaches. A roadmap (panel 3) outlines a framework and call to action for achieving these goals. When all of these strategies are brought to bear, drugs and diagnostics will together make a powerful combination.

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Table 1

Advantages and *disadvantages* of centralized and peripheral DST*

Issue	DST in centralized/reference labs (status quo for most high burden countries)	DST in peripheral settings (e.g. microscopy centers or district labs)
Technology requirements	Centralized labs allow for deployment of high throughput, sophisticated assays (e.g., microarrays, DNA sequencing, beacons, RT-PCR); these may be better suited for assaying many mutations and more drugs.	*This setting might constrain technology to simpler platforms which may not be ideal for new drugs or the addition of more drugs/mutations. The accompanying sample preparation technique must not require a BSL-3 laboratory.*
Cost	Centralized DST may be only for subpopulations of patients, so volume and therefore costs are reduced. Samples can be batched to further increase cost efficiency.	*DST assays for the periphery may be more expensive and not cost efficient (lower test volume). Overall cost of tuberculosis diagnosis may increase and health systems may be unwilling to make such big investments, unless MDR-TB prevalence is very high.*
Quality	It is much easier to ensure quality testing and reliable results in a limited number of centralized labs.	*Unless very simple or automated, DST in the periphery will require extensive quality assurance, training, and personnel.*
Timeliness and use of results	*Major issues with turnaround times. Losses to follow up are high – both with samples sent, and with patients who never come back for results. DST results are often not reviewed when they become available, and a good percentage of results never get reported or used.*	If universal DST is needed at the time of tuberculosis diagnosis, then it has to be done in peripheral settings where most tuberculosis cases are diagnosed. Rapid turnaround and lower losses to follow-up will mean doctors can actually act on the DST results and modify treatment decisions. Likely to pick up drug- resistant tuberculosis much earlier, before transmission occurs to a larger number.
Sample transport and reporting system	*Requires good sample transport and reporting system, which is not available in many settings.*	No requirement for an extensive sample transport and reporting system.

* Advantages are in regular text; disadvantages are in *italics*.

Panel 1

Key messages

New drug regimens and new diagnostics for tuberculosis, including drug susceptibility testing, are bringing great excitement. However, their full potential will only be realized if strategies are aligned to promote co-introduction.
The ultimate goal is tuberculosis treatment that is based on full information about the drug susceptibility of the infecting strain, for all patients. However, at least in the short term and in the setting of constrained resources, less comprehensive drug susceptibility testing may be considered. Potential gains from any testing strategy must be balanced with the associated costs, complexity, and predicted loss to follow-up.
Drug susceptibility testing and drug resistance surveillance are particularly important for the existing and repurposed drugs, such as pyrazinamide and fluoroquinolones, that are being tested in potential first line regimens and for which resistance already exists.
Drug susceptibility tests should be rapid in order to maximize the retention of people with tuberculosis and their prompt treatment with effective regimens, and thus to minimize the generation and spread of drug-resistant disease. Due to the slow growth of *Mycobacterium tuberculosis*, a rapid drug susceptibility test will likely need to detect molecular, rather than phenotypic, correlates of resistance.
To improve the basis for molecular tests, further research is needed to fully establish the genetic basis for resistance to existing and new drugs and to correlate each mutation with clinical impact. Surveillance is also needed to establish the background level of resistance to existing and new drugs.
This information can be used as inputs by multiple groups: modelers will evaluate the potential effectiveness of different scenarios of drug and diagnostic introduction; product developers can better define what product specifications are required; and country programs and providers can better assess whether, and how, to adopt the various new products.

Panel 2

What diagnostics developers require beyond target product profiles[60*]

Potential market size
Size of the target population
Market reach of competing DST technologies
Diagnostic algorithms used now and in the future; current and future TB treatment landscape
Segmentation of markets by income and by peripheral versus centralized methods
Projected scale-up dynamics
Practical steps required
Sources of funding and technical support, especially for validation trials
Whether validation trails can address only accuracy or must also show clinical impact
Requirements for regulatory and policy approvals
Potential procurement and scale-up challenges at the country level

*A more detailed list of FAQs by TB test developers is available at www.tbfaqs.org

Panel 3

Roadmap for action

Short term
Identify all mutations in *Mycobacterium tuberculosis* that occur with reasonable frequency and that result in resistance to existing and new drugs. Priority should be placed on resistance to “backbone” agents and on obtaining information from clinical samples that are accompanied by treatment outcome data.
Develop a collection of sequenced sensitive and resistant strains that can be used to evaluate new DST assays.
Use modeling to define which strategies for deployment of DST have the greatest potential population- level impact and cost-effectiveness. The various strategies would include: (i) different DST assays that vary in their speed, sensitivity and specificity, cost, and technical specifications; and (ii) different DST algorithms, used in the context of various baseline resistance levels.
Conduct surveillance of moxifloxacin resistance among new tuberculosis patients and of pyrazinamide resistance among new and MDR-TB patients.
Conduct operational research to assess and optimize systems for sputum transport and reporting results (including prompt initiation of treatment in response).
Develop clear target product profiles to guide diagnostics developers regarding the necessary product specifications and likely market demand.
Conduct analyses of the TB diagnostics market size and potential, to inform investment decisions by test developers.
Medium term
Use existing diagnostics platforms to develop, field test, and commercialize DST assays, in particular for fluoroquinolones and pyrazinamide, that can be implemented at the subdistrict level.
Monitor for clinical resistance generated during the roll-out of new tuberculosis drugs (i.e., new chemical entities) and determine the molecular basis for such resistance.
Refine models of long-term impact based on early surveillance data during roll-out of novel regimens.
Develop DST assays for new tuberculosis drugs and use them to conduct ongoing surveillance.
Develop and strengthen systems for using next generation sequencing for tuberculosis drug surveillance.
Long term
Develop new diagnostic platforms that are rapid, inexpensive, and can be implemented at the subdistrict level.
Develop a “universal regimen” for tuberculosis that has at least three novel chemical entities and that therefore minimizes the need for DST while treating all forms of tuberculosis.