INTRODUCTION OF A NEW RAPID DIAGNOSTIC TOOL AND SHORT-COURSE REGIMEN FOR DRUG-RESISTANT TUBERCULOSIS IN SOUTH AFRICA

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Executive summary

Multidrug-resistant tuberculosis (MDR-TB) was declared a public health crisis by the World Health Organization (WHO) and has been characterised by prolonged therapies and diagnostic delays in drug resistance detection, both leading to poor outcomes. New WHO recommendations state that in patients with rifampicin-resistant or multidrug-resistant TB who have not been previously treated with second-line drugs and in whom resistance to fluoroquinolones and second-line injectable agents has been excluded, or is considered highly unlikely, a shorter MDR-TB regimen of 9-12 months may be used instead of the conventional regimen. The WHO also recommends the use of the GenoType MTBDR*sl* (SL-LPA) for patients with confirmed rifampicin-resistant TB or MDR-TB as the initial test to detect resistance to fluoroquinolones and the second-line injectable drugs, instead of phenotypic culture-based drug-susceptibility testing. The high TB-HIV co-infection rates and background second-line TB resistance rates in South Africa could potentially undermine the effectiveness (success and relapse rates) of the shorter regimen if it is not introduced prudently. Use of rapid molecular technologies is essential to the introduction of the shorter regimen, including both the SL-LPA and FL-LPA. The latter is required for triaging the use of high-dose isoniazid and ethionamide in the shorter regimen. Any evidence of resistance to both is an additionally-recommended exclusion criterion for use of the shorter regimen apart from any fluoroquinolone or second-line injectable drug resistance detected. The introduction of the shorter regimen and SL-LPA no doubt improves the detection and management of drug-resistant TB in SA and complements gains being made with the use of newer therapeutic agents in the programme.

Background

Multidrug-resistant tuberculosis (MDR-TB) has been declared a global public health crisis by WHO and in the latest global report an estimated 580 000 incident cases occurred in 2015 alone. South Africa is one of 30 high MDR-TB-burden countries, contributing an estimated 20 000 cases in 2015.[1]

Conventional MDR-TB therapy is prolonged – treatment lasts at least 18 to 24 months and includes an intensive phase with an injectable agent for up to 8 months. Although the time to diagnosis of first-line resistance using molecular tools has reduced from months to days, this is not the case for second-line resistance, which requires slower phenotypic methods. Treatment outcomes for MDR-TB are poor, with only 50% of cases achieving successful outcomes. This reduces to only 20% for extremely drug-resistant TB (XDR-TB).[1] These poor outcomes may be attributed to the long duration of therapy and the intolerability of

drugs included in the treatment regimen. Both contribute to higher 'lost to follow-up' and treatment interruption rates. Additionally, delays in diagnosis, which often depends on microbiological confirmation, is an aggravating factor.

In May 2016, the World Health Organization (WHO) issued recommendations for a shortened MDR-TB treatment regimen (SR)[2] based on observational studies from 10 countries including Bangladesh (n=493), Swaziland (n=24), Uzbekistan (n=65) and seven other sub-Saharan African countries (n=408). Among those with MDR-TB and without previous second-line therapy, successful patient outcomes for those on the 9-12 month regimen were higher than those on the conventional long regimen - 84% (95% CI 79–87%) versus 62% (95% CI 53–70%), respectively.[2] Of the 39% of patients followed up after 12-18 months of treatment completion on the SR, none had a relapse and all were culture negative. These positive findings led to the latest WHO guidelines recommending the use of the SR. The WHO recommendation states that in patients with rifampicin-resistant or multidrug-resistant TB, who have not been previously treated with second-line drugs and in whom resistance to fluoroquinolones (FLQ) and second-line injectable agents (SLID) has been excluded or is considered highly unlikely, a shorter MDR-TB regimen of 9-12 months may be used instead of the conventional regimen.[2] This SR comprises an intensive phase of 4-6 months followed by a continuation phase of 5 months. The treatment regimen comprises 7 drugs during the intensive phase, also known as the injectable phase: kanamycin, moxifloxacin, ethionamide, ethambutol, clofazamine, pyrazinamide and high-dose isoniazid. The continuation phase comprises 4 drugs i.e. moxifloxacin, clofazamine, pyrazinamide and ethambutol.

An important prerequisite for the implementation of the shortened regimen is the need for rapid second-line drug susceptibility testing to exclude resistance to the two core agents (FLQ and SLID). The turn-around time for current phenotypic drug susceptibility results is extremely lengthy, requiring 6 – 8 weeks or longer for results to be available, and limits its use for early regimen selection. The need for early regimen triaging is of even greater importance in light of emerging evidence of cross-resistance between clofazimine – a core drug in the SR – and bedaquiline (BDQ), an increasingly-used drug for pre-XDR and XDR-TB cases. Thus delays in the diagnosis of these more resistant forms of TB with exposure to clofazimine in the SR could compromise the next-level BDQ-based regimens used to treat such cases. A new version of the GenoType MTBDR*sl* line probe assay Version 2.0 (Hain Life Sciences, Nehren, Germany) was released in 2015 and offers a potential to address the need of rapid detection of pre-XDR and XDR, being able to identify resistance to FLQ and SLID in days for smear-positive cases, while smear-negative cases may need repeat testing on culture-positive isolates. The genetic targets for resistance determination are *gyr*A and *gyr*B for the FLQ class, and the *rrs* and *eis* promoter for the SLID class. The *eis* promoter target in the latest version of the assay provides improved sensitivity for detecting kanamycin resistance – a drug which is widely used in South Africa for RR/MDR-TB treatment. In 2016 the WHO reviewed data available for the new assay and endorsed the GenoType MTBDR*sl* line probe assay Version 2.0 (Hain Life Sciences, Nehren, Germany) as a rapid initial test to be performed on patients with confirmed RR/MDR-TB in place of second-line DST to detect resistance to FLQ and SLID.[3]

This test can be performed on clinical isolates or directly on sputum samples, eliminating the delays associated with culture. The sensitivity and specificity on smear-positive samples was determined to be 93% and 98.3% for FLQ and 88.9% and 91.7% for SLID.[4] The WHO recommends the use of the SL-LPA for patients with confirmed rifampicin-resistant TB or MDR-TB as the initial test to detect resistance to fluoroquinolones and the second-line injectable drugs, instead of phenotypic culture-based drug-susceptibility testing (DST).

Critical review and significance

The WHO recommendations are an important advancement in the current management of rifampicin-resistant (RR) and MDR-TB and will likely reduce problems concerning adherence. However, there are several issues that need to be considered and a critical review of the data needs to be performed to inform application of these recommendations in the South African context. The successful drug-resistant TB treatment outcomes reported in the non-intervention arms of the studies have been higher than that reported routinely in South Africa (62% versus 48%). This is probably related to the definitions applied, as pre-XDR cases are usually included amongst MDR-TB cohorts for WHO reporting, while these studies have been applied in patients without fluoroquinolone or injectable resistance. The low prevalence of HIV in these studies may have also resulted in better outcomes and low relapse rates. Additionally, the results from the South Africa TB Drug Resistance Survey 2012-14 (DRS) showed high levels of resistance to second-line agents, raising further concerns. The prevalence of resistance among MDR-TB for pyrazinamide was 59% (49.0%-69.1%) and for ethambutol was 44.1% (30.2%-58.0%). Both drugs are included in the continuation phase with moxifloxacin and clofazimine. Ethionamide, having similarly high resistance levels to ethambutol, is added in the intensive phase. It should be noted that the DRS prevalence data among MDR-TB includes the subsets of pre-XDR/XDR, and if these cases were excluded the prevalence would be lower. Clofazimine was not tested but prevalence rates are expected to be low as this drug has been primarily reserved for use in treating pre-XDR/XDR cases, which are excluded from the SR.

The high prevalence of HIV and TB drug resistance raises questions as to the applicability of the SR in South Africa. The inclusion of Swaziland data, which shares a very similar epidemiology to South Africa, only contributed less than 3% of the sample analyzed. More information has however become available since the WHO announcement, with data from close to 100 patients now available (unpublished) with, encouragingly, similarly good outcomes to those of other countries. In the local context, the choice of continued use of high-dose isoniazid and ethionamide for the full duration of treatment is strongly recommended. At least 4 effective TB drugs are required to treat MDR-TB and, in the current SR when used in combination with screening for FLQ and SLID resistance, these two drugs, with clofazimine being the third, will have a high likelihood of effect. The choice of a 4th effective drug is expected from ethambutol, high-dose isoniazid or ethionamide. Pyrazanimide is an important sterilizing agent, is known to have a positive contribution to shortened regimens, and is included irrespective of resistance. The latter is also often a challenge to accurately determine *in vitro* and as such, is best included.

The high specificity of the SL-LPA assay implies that results of resistance testing can be acted upon and this is also the WHO recommendation. There are, however, certain limitations as the test provides resistance determination by drug class and not for individual drugs. In the case of FLQ, this is based on ofloxacin and has shown good correlation. However, moxifloxacin, a newer generation fluoroquinolone, which is widely used for treatment, is likely to have some effect against strains with mutations that confer lower levels of resistance, and could thus potentially be used with effect by applying a higher dosage. Despite this limitation with the SL-LPA over-estimating low-level resistance, it does provide a conservative approach by excluding these patients from SR. For the SLID, correlation with kanamycin resistance, which is the core drug for adult treatment, was also very high and would appropriately exclude such cases with resistance. On the opposite end are concerns around the sensitivity of the assay in identifying resistance, which has been shown to be variable. The assay could miss approximately 15% of each class of resistance and the implications are that some of these patients may be started on an SR. Although this appears to be a high proportion of cases, based on the most recent DRS, the prevalence of resistance to each of these drug classes among MDR-TB patients was 13%, meaning that the vast majority of cases would be susceptible and of those resistant (13%), only a subset (15% of the 13%) would be missed. Testing all cases phenotypically to identify such a small proportion may not be feasible, and it would thus be prudent to minimize this group by also including prior

second-line drug exposure history, especially those with unsuccessful outcomes in that episode, as an exclusion criterion. Lastly, clinical response and culture conversion will also serve as an important indicator for subsequent phenotypic resistance testing.

Public health significance and applicability

Following the WHO recommendations, a series of consultations followed with relevant stakeholders and a revised laboratory-clinical algorithm was developed (Table 1) while the new revised guideline is still in development. An important requirement for the implementation was the need for close alignment between the laboratory tools and treatment decisions. Furthermore, current algorithms in place, including the use of bedaquiline (a new agent for pre-XDR and XDR-TB patients, as well as selected MDR-TB patients) and FL-LPA mutation profiles used to guide therapeutic decisions, needed to be incorporated. Patients with *katG* mutations which are likely to have high levels of isoniazid resistance, and high-dose isoniazid may not be effective in some individuals, based on their host genetics. Thus, due consideration should be given to exclude this drug in the regimen. However, the possibility of *in vivo* synergy between isoniazid and clofazamine has been suggested previously and is another consideration to continue high-dose INH despite the presence of a *katG* mutation. Patients with an *inhA* mutation are likely to have ethionamide resistance, and would not have this agent included in their regimen, which apart from the resistance, is further justified due to its poor patient tolerability profile. The new algorithm incorporates these elements as well as the new WHO recommendations. Thus, in the latest iteration of the algorithm, patients with any FLQ or SLID resistance, and those with mutations in both *katG* and *inhA* would be excluded from SR. The latter criterion was important, as a double mutation would imply the loss of two agents thus compromising the SR. The loss of one of the latter two agents is not an exclusion and is different from the WHO recommendations. The rationale for this is that the absence of either one of these mutants implies susceptibility to the drug (i.e. high-dose INH or ethionamide) and thus at least four active drugs are available to complete therapy. All other patients would be referred to the next level of care for a decision on an individualised regimen and the use of BDQ. As testing accuracy is not absolute it was agreed that patients with prior multidrug-resistant therapy with line probe assay results indicating eligibility for the Short MDR Regimen, would need phenotypic DST and the case closely followed up, though it was appreciated that such cases are likely to be uncommon.

Table 1. Conceptual framework for the revised rifampicin-resistant/multidrug-resistant TB algorithm incorporating second-line line-probe assay

Step	Patient Status	Action
1	Rifampicin-resistant TB	Consider patient for SR, submit sample for DR-TB reflex test and complete baseline assessments
2	Is patient eligible with no contraindications present	If yes, start SR and follow-up LPA results
3	LPA – second-line result	
	3.1. Resistant to fluoroquinolones	Stop SR and refer to next level of care
	3.2. Resistant to injectable agent	Stop SR and refer to next level of care
4	LPA - first-line result	
	4.1. Isoniazid-resistant	
	katG mutation present	Continued use of high-dose isoniazid uncertain
	inhA mutation present	Stop ethionamide and continue SR
	Both *katG* and *inhA* mutations present	Stop SR and refer to next level of care
	4.2. Isoniazid-sensitive	Continue SR

SR: Short regimen, LPA: Line probe assay

Operational consideration included both clinical and laboratory indicators. The SL-LPA uses the same infrastructure as for the LPA first line (FL-LPA), which is widely used (dating back to 2009) and makes the implementation easier. The reduction in the use of the FL-LPA since the introduction of GXP in 2011 resulted in underutilization and this spare capacity is available for the introduction of the new assay. Testing using the new SL-LPA began on 1st of January 2017 and by the end of April 2017, 4282 samples were tested. Uptake has been good with almost all districts having patient samples tested and volumes increased month on month since January. Testing is now linked to a 'super-set' of tests for baseline assessment of all new RR/MDR-TB patients starting the SR, which includes smear microscopy, culture and both first- and second-line LPAs. In addition, if any resistance to FLQ or SLID is detected, the designated laboratories for second-line TB testing would proceed to perform phenotypic DSTs to moxifloxacin at two concentrations, capreomycin, and linezolid to aid formulation of an individualised regimen.

An important issue for clinical implementation is the drug availability of clofazimine and the additional clinical assessments (e.g. audiology, ECG etc.). For the latter, these are well established in the historic DR-TB initiation sites but are now being expanded to decentralised sites across the country and are at variable stages of implementation. The issue around clofazamine accessibility is, however, a bigger one as it is a core drug for the SR and is thought to have sterilizing activity that has led to success in reducing treatment duration. To date the drug has been accessed through the Section 21 regulatory process on a named-patient basis. This is not sustainable long-term and efforts are underway to address the challenge. Clofazimine is currently not officially registered for MDR-TB therapy in South Africa and due to the low uptake and associated costs, this has not materialised. The WHO has recently added the drug to the Essential Medicines List (EML) but the local registration through the Medicines Control Council still needs to be completed by the manufacturer. In the interim, several provinces have already procured stock through the existing procedure and have begun initiating patients on SR. The roll-out of the SR has been accompanied by a package of training of doctors and nurse initiators at the decentralised sites that are now in excess of 600 nationally.

There are some important gaps not fully addressed in the guidelines which are still being considered for the local context. Pregnant women and patients with extra-pulmonary TB (EPTB) would not normally be eligible for SR, but there is a rationale to consider uncomplicated EPTB for SR with a longer duration as is done for drug-sensitive TB. For pregnant women, efficacious drug substitution for the injectable may be justified in the SR as is already practiced for the current standard MDR regimen. Cases where testing is not performed or testing fails, and patients are put on SR, will require careful consideration in the final guidelines. Furthermore, patients on SR that have failed or are lost to follow-up are another group for which guidance needs to be developed. The SR and SL-LPA are new innovations and close monitoring with regular reviews will be essential to chart the best course for these emergent issues.

Conclusion

The introduction of the SR and SL-LPA no doubt provides important steps to improving the detection and management of drug-resistant TB in South Africa, and complements gains being made with the use of newer therapeutic agents. Anecdotally, responses from laboratorians and clinicians has been very positive with pre-XDR and XDR cases now being identified in under a week, which has revolutionised diagnostics in the DR-TB program. Although the clinical efficacy of SR may not be dramatic, the public health benefits to patients with a regimen whose duration is similar to the current one for susceptible TB is likely to reduce loss to follow-up rates, and improve overall outcomes. Furthermore, this will now set a new standard for DR-TB management with shorter regimens in the future. It is still early but the expectation is that these new changes will help take South Africa closer to achieving the goals of the END TB Strategy.

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