CURRENT STANDARD DRUG REGIMENS FACILITATE THE EVOLUTION OF EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS: Recommendations for improvements

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Background

- Tuberculosis (TB) is caused by Mycobacterium tuberculosis. Globally, the disease kills more than 1.3 million people each year (1).
- South Africa has one of the highest TB incidence rates in the world, with almost 1% of the population developing TB every year. This is over three times more than the threshold value for the highest incidence rate category (≥0.3%) as defined by the World Health Organization (WHO) (1).
- TB is a leading cause of death in HIV-infected individuals and the emergence of drug resistance, in particular, complicates TB treatment for this patient group (1).
- Of all notified TB cases in South Africa in 2008, 20.2% showed resistance to isoniazid (H) and nearly half of these (9.6% of all cases) were multidrug-resistant TB (MDR-TB), a form which is resistant to the two most potent first-line anti-TB drugs: H and rifampicin (R) (2). A nationwide survey in 2002 identified a rate of MDR-TB of 3.1% among all TB cases, thus indicating a dramatic increase in the proportion of MDR-TB in recent years (3).
- The South African national TB control program has the second largest budget (US $352 million in 2008) worldwide, after the Russian Federation, with approximately 70% of it being allocated to the management of MDR-TB (4).
- Cases of virtually untreatable extensively drug-resistant TB (XDR-TB) have been described in various settings in South Africa, with a nosocomial outbreak of XDR-TB in KwaZulu-Natal having received international media attention (5). XDR-TB is defined as MDR-TB with additional resistance to a fluoroquinolone (mostly ofloxacin (Ofx)), an aminoglycoside (Km or Am), Z, E or terizidone/cycloserine (Trd/Cs) and ethionamide (Eto) (12).
- Contrary to previous dogma, there is mounting evidence that the majority of MDR-TB in South Africa is due to the transmission of drug-resistant strains of M. tuberculosis rather than the acquisition of resistance in non-compliant patients (6-10). In the absence of drug susceptibility testing (DST) at the onset of TB treatment, undiagnosed MDR-TB cases are at risk of acquiring additional resistance, leading to XDR-TB treatment in South Africa.

Diagnosis of drug-resistance

- DST in South Africa is generally performed using the liquid culture-based Mycobacteria Growth Indicator Tube (MGIT) system, BACTEC™ MGIT960 (BD Diagnostic Systems) (12).
- In many settings, culture-based phenotypic DST for first-line drugs has been replaced by molecular genotypic DST using the WHO endorsed GenoType® MTBDRplus assay (Hain Lifescience). This assay detects H resistance-conferring mutations in the katG gene and the inhA promoter and R resistance mutations in the rpoB gene (15).
- Of clinical importance is the observation that mutations in the inhA promoter usually confer low-level resistance to H and cross-resistance to Eto, while mutations in katG, for example, at codon 315, exclusively confer high-level resistance to H but no cross-resistance to Eto (16). Other drug-resistance mutations that confer resistance to anti-TB first-line drugs are mutations in rpoB for R, mutations in pncA for Z and mutations in embB for E (16). A database of most of the known drug-resistance mutations is accessible at www.TBDrugDB.com.

Research results

The evolution of XDR-TB in South Africa
Standardised second-line treatment regimens in South Africa, which were in use from 2002 until recently, assumed that resistance to Z and
E is rare in MDR-TB. This assumption was partially based on inaccurate culture-based DST results and challenged by molecular surveillance data (14). In a study in the Western Cape Province, we showed that in a sample of drug-resistant strains tested (resistant to one or more drugs other than Z), 53.5% were resistant to Z (17). In another study, 57.5% of all MDR strains also showed mutations in the embB gene that conferred resistance to E (13). Moreover, previous second-line regimens failed to recognise the finding that mutations in the inhA promoter, which occur at high frequency among MDR- and XDR-TB patients (Figure 1) (18), confer cross-resistance to Eto (16,19).

With the use of these second-line regimens, a large percentage of MDR-TB cases were essentially treated with only two effective drugs (Km/Am and a fluoroquinoline), of which Km/Am was only used during the intensive treatment phase. This resulted in mono-therapy and the rapid evolution of fluoroquinolone resistance and pre-XDR-TB during the continuation phase (14). These results prompted a change in DoH guidelines to replace E with TdR/Cs in standardised MDR-treatment regimens. However, the frequent occurrence of Z resistance, and the possibility of cross-resistance between H and Eto, still have not been taken into consideration and may fuel the emergence of XDR-TB (14).

Strains with an inhA promoter mutation are overrepresented among XDR-TB cases MDR strains that acquired an inhA promoter mutation will be exposed to a second-line treatment regimen with one less effective drug if Eto resistance remains undetected. There is a strong possibility that this will increase the likelihood of such strains developing XDR-TB compared to strains not harboring an inhA promoter mutation. To test this hypothesis, we investigated the frequency of drug-resistance mutations among MDR, pre-XDR and XDR TB cases in the Western Cape, Eastern Cape and KwaZulu-Natal provinces. We demonstrated that the presence of inhA promoter mutations are strongly associated with XDR-TB in the Western and Eastern Cape provinces (Figure 1) (18). In a separate study, whole genome sequencing of nine XDR strains from different settings in KwaZulu-Natal revealed that each one harbored an inhA promoter mutation (20). Also, we demonstrated that strain diversity decreases significantly from MDR- to XDR-TB cases. Only a handful of strains, all of them characteristically associated with an inhA promoter mutation, contribute to the emergence of XDR-TB (Figure 2) (6,9,10). Moreover, there is accumulating evidence that MDR-TB in South Africa is primarily due to the transmission of drug-resistant strains, and to a lesser extent caused by the acquisition of resistance (6-10). Taken together, molecular epidemiological data from the Western Cape, Eastern Cape and KwaZulu-Natal provinces suggests that XDR-TB is mostly caused by a few successfully transmitting drug-resistant strains, which characteristically harbor an inhA promoter mutation.

**Recommendations**

1. **The availability of molecular drug susceptibility testing can provide important guidance for adapted treatment regimens**

Molecular methods such as the GenoType® MTBDRplus assay are becoming widely available and provide important information on (a) the likely level of H resistance and (b) cross-resistance between H and Eto. Treatment guidelines should utilise this important information in order to optimise the treatment of patients with drug-resistant TB (Figure 3).

2. **The presence of inhA promoter mutations identify MDR-TB patients at higher risk of XDR-TB development**

The higher proportion of inhA promoter mutations in XDR-TB compared to MDR-TB cases suggests that patients with MDR-TB isolates harboring this marker are at greater risk of developing XDR-TB, although this is probably related to suboptimal MDR treatment.

3. **In the presence of an inhA promoter mutation, patients must not receive treatment with Ethionamide**

Mutations in the inhA promoter confer low-level resistance to H and resistance to Eto, which is a structural analogue of H (16). Using Eto in second-line regimens for the treatment of MDR-TB harboring an inhA promoter mutation cannot be justified since it is ineffective, adds cost and only increases drug-related adverse events (Figure 3) (18,19).

4. **For strains that show a mutation in the inhA promoter but not in the katG gene, inclusion of high-dose H in the treatment regimen should be considered**

Since mutations in the inhA promoter only confer low-level resistance to H (16), continued use of high-dose H (15mg/kg) has value and strengthens the treatment regimen (18,19). High-dose H cannot be relied upon, but should be considered as there could be other mechanisms conferring high-dose H resistance, which remain undetected by the MTBDRplus assay (Figure 1).

5. **E and Z should not be considered effective drugs in second-line treatment regimens**

Due to the often undetected resistance of MDR strains in South Africa to Z and E, these drugs may be added, but cannot be considered effective in second-line treatment regimens (14).

6. **Culture-based DST remains indispenable for surveillance purposes and to assist interpretation of molecular techniques**

In order to achieve the highest possible sensitivity for the detection of drug-resistant isolates and to monitor possible shifts in the frequency of resistance mutations under treatment pressure, culture-based phenotypic DST should not be discontinued. Ongoing surveillance is essential from a public health perspective and also assists the interpretation of molecular drug-resistance testing in individual patients. It is conceivable that exclusive use of genetic tests could lead to the selection and spread of resistant strains with properties that remain undetected by the molecular identification methods currently in use (Figure 1).

7. **Drug regimens must be determined by DST results**

To ensure correct therapy of TB patients, DST should be performed at treatment onset and the regimen of choice determined by the DST result. Figure 3 gives treatment recommendations contingent on molecular DST results as provided by the MTBDRplus assay. The recommendations are that current highly standardised treatment regimens should be replaced by more individualised medication. Note that susceptibility testing of E, S and second-line drugs, as well as the management of potential adverse events, could require further adaptation of drug regimens.

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**Figure 1.** The proportion of MDR *sensu stricto* (MDR s.s.; excluding identified XDR isolates) and XDR-TB isolates with an *inhA* promoter mutation in the Western Cape and Eastern Cape provinces. The presence of mutations in the *katG* gene has not been assessed for isolates from the Eastern Cape province. Only others: Isolates for which none of the tested mutations have been detected; *katG/inhA* promoter: Isolates with mutations detected in the *katG* gene but not in the *inhA* promoter; *inhA* promoter and *katG*: Isolates with mutations detected in the *inhA* promoter and in the *katG* gene; *inhA* promoter*katG*: Isolates with mutations detected in the *inhA* promoter but not in the *katG* gene.

**Figure 2.** Frequency distribution of families of strains stratified by drug-resistance group for three South African provinces. The proportion of isolates belonging to the five most prominent families of strains is indicated for MDR *sensu stricto* (MDR s.s.; excluding identified pre-XDR and XDR isolates), pre-XDR and XDR cases. The proportion of typical and atypical Beijing strains among the Beijing strains in the Western and Eastern Cape is indicated.
**Figure 3.** Proposed guidelines for an adapted treatment of TB patients in a South African context on MTBDRplus-based DST results. Additional DST results and management of adverse effects may demand further adaptation of treatment regimens. In addition to DST for H or R, O, X and Am, phenotypic DST for E and S may be performed upon request at the National Health Laboratory Service (NHLS) of South Africa. DST for Z is currently not done at NHLS. Genotypic DST for Z and E could provide more reliable results than culture-based techniques but cannot be performed using present-day commercial products and therefore require the setup of in-house systems. Standard drug codes were used to describe drug regimens with numbers indicating time (in months) of medication with a given combination of drugs (21). H: Isoniazid; R: High-dose isoniazid; R: Rifampicin; Z: Pyrazinamide; E: Ethambutol; S: Streptomycin; Ltb: Levofloxacin; Otb: Ofloxacin; Km: Kanamycin; Am: Amikacin; Eto: Ethionamide; Td: Terizidone; Cs: Cycloserin; PAS: Para-aminosalicylic acid.

If culture-based phenotypic DST is used not enabling the differentiation between inhA promoter and katG gene mutations, this regimen may be used for MDR-resistant TB.

The treatment duration indicated is the minimal duration. The duration of intensive phase therapy should be at least six months or four months after culture conversion if conversion occurs later than after two months. Continuation phase therapy should be at least 12 months. However, a total treatment duration of 18 months after culture conversion is recommended.

If culture-based phenotypic DST is used not enabling the differentiation between inhA promoter and katG gene mutations, this regimen may be used for MDR-TB provided that susceptibility to at least four of the indicated drugs is highly likely.

**References**