



Short communication

Whole-genome sequencing of multidrug-resistant *Mycobacterium tuberculosis* isolates from Myanmar

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ABSTRACT

Drug-resistant tuberculosis (TB) is a major health threat in Myanmar. An initial study was conducted to explore the potential utility of whole-genome sequencing (WGS) for the diagnosis and management of drug-resistant TB in Myanmar. Fourteen multidrug-resistant *Mycobacterium tuberculosis* isolates were sequenced. Known resistance genes for a total of nine antibiotics commonly used in the treatment of drug-susceptible and multidrug-resistant TB (MDR-TB) in Myanmar were interrogated through WGS. All 14 isolates were MDR-TB, consistent with the results of phenotypic drug susceptibility testing (DST), and the Beijing lineage predominated. Based on the results of WGS, 9 of the 14 isolates were potentially resistant to at least one of the drugs used in the standard MDR-TB regimen but for which phenotypic DST is not conducted in Myanmar. This study highlights a need for the introduction of second-line DST as part of routine TB diagnosis in Myanmar as well as new classes of TB drugs to construct effective regimens. © 2016 The Authors. Published by Elsevier Ltd on behalf of International Society for Chemotherapy of Infection and Cancer. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Myanmar is one of the 22 high-burden tuberculosis (TB) countries, with a high prevalence of multidrug-resistant TB (MDR-TB) [1]. Rapid detection is essential to treat patients with drug-resistant TB. Yet conventional drug susceptibility testing (DST) takes several weeks owing to the culturing requirement and subsequent laborious phenotypic testing. Consequently, molecular DST using the Hain GenoType MTBDRplus v.2.0 (Hain Lifescience GmbH, Nehren, Germany) and, more recently, the Cepheid GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA) has been established in Myanmar. However, these assays only interrogate the most

frequent resistance mutations for a limited number of antibiotics. Whole-genome sequencing (WGS) has the potential to overcome this limitation and can be used to identify patients with drug-resistant TB [2–4]. Whilst WGS is being considered for routine diagnosis and management of drug-resistant TB in well-resourced, low-TB burden settings, currently there are no plans for routine implementation in resource-limited, high-TB burden countries. Since it is important that new tools with the potential to improve TB control are adopted as early as possible especially in countries where these tools are needed the most, a preliminary evaluation of the utility of WGS in the diagnosis and management of drug-resistant TB in Myanmar was conducted.

2. Materials and methods

According to Myanmar national guidelines for the management of MDR-TB [5], suspected MDR-TB patients (Table 1) who are sputum smear-positive are referred to the National TB Reference

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Table 1
Treatment category by type of tuberculosis (TB) patient and recommended treatment regimens [5].

Treatment category	
Cat I	Treatment for new patients with first-line anti-TB drugs (2 months of INH, RIF, EMB, PZA/4 months of INH, RIF)
Cat II	Re-treatment regimen with first-line anti-TB drugs (2 months of STR, INH, RIF, EMB, PZA/1 month of INH, RIF, EMB, PZA/3 months of INH, RIF, EMB)
New	A patient who has never had treatment for TB or who has taken anti-TB drugs for <1 month
Close contact	Close contacts of MDR-TB patients who develop active TB
Failure	A patient previously treated for TB who is started on a re-treatment regimen after previous treatment has failed
Relapse	A patient who was previously declared cured or treatment completed and is diagnosed with bacteriologically-positive (sputum smear or culture) TB
Standard MDR-TB regimen	6 months of AMK, PZA, LFX, ETH, DCS/18 months of PZA, LFX, ETH, DCS ^a

INH, isoniazid; RIF, rifampicin; EMB, ethambutol; PZA, pyrazinamide; STR, streptomycin; MDR, multidrug-resistant; AMK, amikacin; LFX, levofloxacin; ETH, ethionamide; DCS, cycloserine.

^a Para-aminosalicylic acid will be added to the regimen if a resistance mutation in the *inhA* promoter is detected with MTBDRplus or if the patient cannot tolerate ETH or DCS.

Laboratories in Yangon and Mandalay for genotypic testing with the Hain GenoType MTBDRplus v.2.0 as well as phenotypic DST. Sputum specimens are decontaminated and are then inoculated onto Löwenstein–Jensen medium for culturing and phenotypic DST of isoniazid, rifampicin, ethambutol and streptomycin [6]. The MTBDRplus is performed according to the manufacturer's instructions and DNA is extracted as described previously [7]. The National Reference Laboratories in Myanmar do not currently perform DST of second-line drugs or pyrazinamide as part of routine diagnosis of drug-resistant TB and do not store culture isolates.

DNA of 14 isolates from MDR-TB patients were selected for this study and were further purified using an UltraClean[®] Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA). DNA was sequenced using paired-end 250-bp reads on an Illumina MiSeq using the Nextera[™] XT DNA Kit (Illumina Inc., Hayward, CA). The resulting sequencing data were submitted to the European Nucleotide Archive (PRJEB10037). Using version 1 of PhyResSE with version 27 of the variant list and, where applicable, literature review, resistance genes for the following antibiotics that are commonly used in the treatment of drug-susceptible and MDR-TB in Myanmar were interrogated: rifampicin; isoniazid; ethambutol; streptomycin; ethionamide; pyrazinamide; amikacin; and levofloxacin [8]. In addition, genes involved in para-aminosalicylic acid (PAS) resistance (*folC*, *ribD* and *thyA*) were analysed [2]. PhyResSE was also used for strain classification.

3. Results

3.1. Strain diversity

The Beijing lineage dominated, with 11 of the 14 strains belonging to that particular lineage. The remaining three strains belonged to the East-African Indian and Euro-American lineages (Table 2).

3.2. Genotype–phenotype concordance for drugs that were tested phenotypically in Myanmar

The WGS results for *rpoB*, *inhA* and *katG* were in full agreement with the results of the MTBDRplus (Table 2). Moreover, two *katG* mutations were detected in isoniazid-resistant strains that cannot be detected with the MTBDRplus: G299C in M00011, which is known to be associated with isoniazid resistance; and a frameshift in M00020, which should result in high-level isoniazid resistance [9].

Of the 14 strains, 7 were phenotypically resistant to ethambutol and harboured known resistance mutations in *embB* or mutations that were previously associated with ethambutol resistance. One

phenotypically susceptible strain (M00004) had a known resistance mutation [4,10].

With respect to streptomycin, all but one strain was phenotypically resistant, but genotypic resistance was identified in only 10 of the 13 resistant strains, due to mutations in *rpsL* that are known to be associated with streptomycin resistance or to potential resistance mutations in *gidB* [4]. It was not possible to test whether these discrepancies were due to laboratory error, given that the strains were not stored.

3.3. Genotypic drug susceptibility testing for drugs used in multidrug-resistant tuberculosis treatment

No phenotypic DST results were available for ethionamide, pyrazinamide, amikacin, levofloxacin, PAS and cycloserine. In addition to the two *inhA* mutants, two strains were identified that were likely ethionamide-resistant as a result of a previously described *ethA* mutation [11]. Of the 14 strains, 6 had mutations that are known to be associated with pyrazinamide resistance [12]. Strain M00017 was predicted to be resistant to amikacin as a result of an *rrs* G1484T mutation [13]. Two strains had high-confidence *gyrA* resistance mutations to levofloxacin [14]. Strain M00005 was most likely resistant to PAS as a result of a premature stop codon in *thyA* [15]. Three more strains were potentially resistant to PAS owing to novel mutations in *folC* or *thyA*. No genotypic prediction for cycloserine was performed as the genotypic basis of resistance is poorly understood.

4. Discussion

This is the first study to report WGS data for drug-resistant TB from Myanmar and provides possibilities for incorporating WGS into clinical management of drug-resistant TB in Myanmar. For example, WGS can provide a diagnosis of resistance to multiple drugs more quickly than standard phenotypic DST so that it can be used to guide treatment of highly drug-resistant cases such as extensively drug-resistant TB (XDR-TB) cases. It can also serve as a tool for quality control to monitor laboratory performance. Furthermore, it could be used to understand transmission in a population. A larger study is planned in Myanmar to explore these possibilities given that sequencing costs are reducing rapidly (now less than US\$200 per *Mycobacterium tuberculosis* isolate) and the availability of fully automated analysis is underway.

Predominance of the Beijing lineage in this study confirmed prior findings of MDR-TB in Myanmar [16]. Isoniazid and rifampicin resistance was primarily due to mutations in codon 315 of *katG* and in codon 531 of *rpoB*, respectively, as previously observed [17,18]. The detection of rare mutations in *katG* that cannot be detected with the MTBDRplus highlights the added value of WGS to resolve discrepancies between phenotypic and

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