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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.

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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
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 levo- 
floxacin [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: G298C in M00001, which is known to be associated with isoniazid resistance; and a frameshift in M000020, 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 gldB [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 rrs1 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
Table 2

Summary of patient details and comparison of phenotypic drug susceptibility testing (DST) results, where available, with whole-genome sequencing (WGS) results.

| ID     | Age  | Sex | Type of patient | Genotype | RIF | INH | EMB | STR | ETH | PZA | AMK | LFX | PAS |
|--------|------|-----|-----------------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|        | (years) |     |                 |           | DST | rpoB | DST Genotype | DST embB | DST | pncA | rrs | gyrA |     |
| M00001 | 55   | F   | Cat II, failure | Beijing   | R   | S531L | R     | katG S315T | R   | L402V | R   | rpsL K43R | W119G |
| M00003 | 28   | M   | Close contact (new) | Beijing | R   | S531L | R     | katG S315T | R   | G406D | R   | rpsL K43R | A90V |
| M00004 | 32   | M   | Cat II, failure | Beijing   | R   | S531L | R     | katG S315T | S   | M306I | r,d | rpsL K43R | Q10P |
| M00005 | 36   | F   | Close contact (new) | Beijing | R   | H526Y | R     | katG S315T | R   | G406A | R   | rpsL K43R | A90V |
| M00008 | 75   | M   | Relapse after Cat II | Euro-American | R   | H516V | R     | katG S315T | S   | R     |     |     |     |     |
| M00010 | 24   | M   | Cat II, failure | Beijing   | R   | S531L | R     | katG S315T | S   | M306I | e,d | rpsL K43R | A90V |
| M00011 | 63   | M   | Cat I, failure | Beijing   | R   | S531L | R     | katG G299 | R   | M306I | e,d | rpsL K43R | A90V |
| M00012 | 44   | M   | Cat II, failure | East-African Indian | R   | H526Y | R     | katG S315T | S   | R     |     |     |     |     |
| M00013 | 19   | M   | Cat II, failure | East-African Indian | R   | S531L | R     | katG S315T | S   | M306I | e,d | rpsL K43R | Y103 |
| M00016 | 48   | M   | Relapse after cat II | Beijing | R   | S531L | R     | inhA C-15T | S   | R     |     |     |     |     |
| M00017 | 68   | M   | Relapse after cat II | Beijing | R   | S531L | R     | katG S315T | R   | E504D | R   | rpsL K43R | Q10P |
| M00018 | 63   | F   | Cat II, failure | Beijing   | R   | S531L | R     | katG S315T | S   | R     |     |     |     |     |
| M00019 | 27   | M   | Cat II, failure | Beijing   | R   | S531L | R     | katG S315T | S   | M306I | e,d | rpsL K43R | T142A |
| M00020 | 42   | M   | Relapse after cat II | Beijing | R   | S531L | R     | katG F5, s | S   | inhA C-15T |     |     |     |     |
|        |      |     |                 |           | DST | rpoB | DST Genotype | DST embB | DST | pncA | rrs | gyrA |     |

RIF, rifampicin; INH, isoniazid; EMB, ethambutol; STR, streptomycin; ETH, ethionamide; PZA, pyrazinamide; AMK, amikacin; LFX, levofloxacin; PAS, para-aminosalicylic acid; R, resistant; S, susceptible; FS, frameshift.

The Hain GenoType MTBDRplus v.2.0 (Hain Lifescience GmbH, Nehren, Germany) results for rpoB, katG and inhA were in agreement with the WGS data. Where applicable, alternative genotypic DST assays that could have been used to detect additional resistance mutations are listed, although it should be noted that low-level heteroresistant mutations might be below the detection limits of some of these assays, which are not well understood [20].

a Covered by AID TB Resistance Module STR/AMK/CAP (AID Diagnostika GmbH, Straßberg, Germany).
b Covered by Nipro PZA line probe assay (Nipro Corp., Osaka, Japan).
c Covered by Hain GenoType MTBDRsl v.1.0 (Hain Lifescience GmbH, Nehren, Germany).
d Covered by AID TB Resistance Module FQ/EMB (AID Diagnostika GmbH).
e Covered by Hain GenoType MTBDRsl v.2.0 (Hain Lifescience GmbH).
f Covered by Nipro FQ line probe assay (Nipro Corp.).
g Mutation not covered by MTBDRplus.
h Novel mutation with unknown effect.

i Stop codon.
genotypic results. The discordance between the genotype and phenotypic ethambutol results in this study was in line with previous findings that ethambutol DST is less reproducible than for other first-line drugs [4].

The drugs used in the MDR-TB regimen in Myanmar consist of 6 months of amikacin, pyrazinamide, levofloxacin, ethionamide and cycloserine, followed by 18 months of pyrazinamide, levofloxacin, ethionamide and cycloserine (and PAS if ethionamide resistance is detected) (Table 1) [5]. None of the strains in this study were predicted to be XDR-TB. However, 9 of the 14 isolates were likely resistant to at least one of the drugs in the aforementioned standard MDR-TB regimen. Moreover, four of the strains (M00004, M00013, M00017 and M00019) were likely resistant to two drugs, which would reduce the number of effective drugs to three in the intense phase and to two during the extended phase (three in the case of M00017). For the strains with gyrA mutations, resistance to levofloxacin might have been overcome by replacing levofloxacin with moxifloxacin, to which these mutations generally confer low-level resistance [14]. By contrast, adding PAS to the regimen of the two potentially ethionamide-resistant strains with ethA M1R mutations (according to standard treatment guidelines) might not have been effective if the fopC mutations in both strains also caused resistance. Replacing amikacin with kanamycin or capreomycin in the patient with the rrs mutation would not have been an option as this mutation confers cross-resistance to all of these aminoglycosides [13]. Consequently, novel classes of TB drugs are required in Myanmar to construct appropriate regimens.

This study highlights the need to introduce second-line DST in routine diagnosis in Myanmar to substantially increase the proportion of MDR-TB patients for whom DST is conducted (in 2013, only 4.4% of confirmed MDR-TB cases were tested for a fluoroquinolone and second-line injectable drug) [1]. This could also provide clarity regarding the prevalence of XDR-TB, which is currently unknown despite the first reported case in 2007 [19]. This could be achieved using phenotypic methods or by introducing one of the current commercial genotypic DST assays such as AID Resistance Module FQ/EMB and STR/AMK/CAP (AID Diagnostika GmbH, Straßberg, Germany), MTBDRsl v.1.0 or 2.0 (Hain Lifescience GmbH) and the Nipro FQ and PZA line probe assay (LiPA) (Nipro Corp., Osaka, Japan) [20]. It should be noted, however, that their ability to detect low-level heteroresistance is poorly understood. Moreover, LiPAs are relatively labour intensive and slow. More decentralised testing for XDR-TB would be preferable (e.g. with the XDR cartridge that is currently being developed for the GeneXpert and GeneXpert Omni). At the same time, this study revealed that even if a prompt and accurate DST service is introduced, new classes of TB drugs are urgently required in Myanmar to construct regimens that are sufficiently active to adequately treat drug-resistant TB cases.

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Competing interests

CUK, JP and SJF have collaborated with Illumina Inc. on a number of scientific projects; CUK is a consultant for the Foundation for Innovative New Diagnostics and a technical advisor for the Tuberculosis Guideline Development Group of the World Health Organisation. The Bill & Melinda Gates Foundation and Janssen Pharmaceutica covered CUK’s travel and accommodation to present at meetings. The European Society of Mycobacteriology awarded CUK the Gertrud Meissner Award, which is sponsored by Hain Lifescience. JP has received funding for travel and accommodation from Pacific Biosciences Inc. and Illumina Inc. SJF has received funding for travel and accommodation from Illumina Inc. All other authors declare no competing interests.

Ethical approval

Ethical approval for this study was given by the Research and Ethical Committee of the University of Medicine 1 (Yagon, Myanmar).

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References

[1] World Health Organization. Global tuberculosis report 2014. Geneva, Switzerland: WHO; 2014.
[2] Koser C, Bryant JM, Becq J, Torok ME, Ellington MJ, Marti-Renom MA, et al. Whole-genome sequencing for rapid susceptibility testing of M tuberculosis. N Engl J Med 2013;369:290–2.
[3] Koser C, Ellington MJ, Cartwright EJ, Gillespie SH, Brown NM, Farrington M, et al. Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. PLoS Pathog 2012;8:e1002824.
[4] Walker TM, Kohl TA, Omar SV, Hedge J, Del Ojo Elías C, Bradley P, et al. Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. Lancet Infect Dis 2015;15:1193–202.
[5] National TB Programme Myanmar. Guidelines for the management of multi-drug-resistant tuberculosis (MDR-TB) in Myanmar. National TB Programme Myanmar; 2013.
[6] Kent PT, Kubica GP. Public health mycobacteriology: a guide for the Level III laboratory. US Department of Health and Human Services; 1985.
[7] Miyata M, Santos AC, Mendes NH, Cunha EA, de Melo FA, Leite CQ. Assessment of the quality of DNA extracted by two techniques from Mycobacterium tuberculosis for fast molecular identification and genotyping. Braz J Microbiol 2011;42:774–77.
[8] Feuerriegel S, Schlesener V, Beckert P, Kohl TA, Mocto P, Cirillo DM, et al. PhyResSE: a web tool delineating Mycobacterium tuberculosis antibiotic resistance and lineage from whole-genome sequencing data. J Clin Microbiol 2015;53:1908–18.
[9] Coll F, McNerney R, Guerra-Assuncao JA, Gunn JR, Perdigao J, Vieville M, et al. A robust SNP barcode for typing Mycobacterium tuberculosis complex strains. Nat Commun 2014;5:4812.
[10] Brossier F, Sougakoff W, Bernard C, Petrou M, Adehamy K, Pham A, et al. Molecular analysis of the embCAF locus and embR gene involved in ethambutol resistance in clinical isolates of Mycobacterium tuberculosis in France. Antimicrob Agents Chemother 2015;59:4800–8.
[11] Cambau E, Vireire M, Machado D, Raskine L, Ritter C, Tortoli E, et al. Revisiting susceptibility testing in MDR-TB by a standardized quantitative phenotypic assessment in a European multicentre study. J Antimicrob Chemother 2015;70:685–96.
[12] Mocto P, Cabibbe AM, Feuerriegel S, Casali N, Dróbniński F, Rodionova Y, et al. Mycobacterium tuberculosis pyrazinamide resistance determinants: a multicenter study. MBio 2014;5:e01819–1914.
[13] Maus CE, Pikayats BR, Shimnick TM. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis. Antimicrob Agents Chemother 2005;49:3192–7.
[14] Willby M, Sikes RD, Malik S, Metchock B, Posey JE. Correlation between gyrA substitutions and ofloxacin, levofloxacin, and moxifloxacin cross-resistance.
in Mycobacterium tuberculosis. Antimicrob Agents Chemother 2015;59:5427–34.

[15] Fivian-Hughes AS, Houghton J, Davis EO. Mycobacterium tuberculosis thymidylate synthase gene thyX is essential and potentially bifunctional, while thyA deletion confers resistance to p-aminosalicylic acid. Microbiology 2012;158:308–18.

[16] Valvatne H, Syre H, Kross M, Stavrum R, Ti T, Phyu S, et al. Isoniazid and rifampicin resistance-associated mutations in Mycobacterium tuberculosis isolates from Yangon, Myanmar: implications for rapid molecular testing. J Antimicrob Chemother 2009;64:694–701.

[17] Aung WW, Ei PW, Nyunt WW, Swe TL, Lwin T, Htwe MM, et al. Phenotypic and genotypic analysis of anti-tuberculosis drug resistance in Mycobacterium tuberculosis isolates in Myanmar. Ann Lab Med 2015;35:494–9.

[18] Phyu S, Stavrum R, Lwin T, Svendsen OS, Ti T, Grewal HM. Predominance of Mycobacterium tuberculosis EAI and Beijing lineages in Yangon, Myanmar. J Clin Microbiol 2009;47:335–44.

[19] World Health Organization. Global tuberculosis report 2008. Geneva, Switzerland: WHO; 2008.

[20] UNITAID. Tuberculosis diagnostics technology and market landscape. 3rd ed. UNITAID; 2014.