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Introduction

Drug-resistant tuberculosis (DR-TB) is a major risk to global tuberculosis (TB) control. Multidrug-resistant TB (MDR-TB) was responsible for 480 000 new cases and 190 000 deaths in 2014. Globally, 30% of MDR-TB cases are resistant to either fluoroquinolones or aminoglycosides, which are the main second-line drugs. Although the incidence of DR-TB has been stable over time, this is due to a low diagnosis rate and high fatality rate of 40%. Only 4.9% of notified TB patients underwent drug resistance testing in 2009, although this increased to 30% by 2015.

As more MDR-TB is diagnosed and treated with second-line drugs, there is now increased emphasis on detecting resistance to these drugs. MDR-TB is often treated with standardized drug regimens, including the 9-month regimen recently approved by the World Health Organization (WHO). The rapid detection of second-line drug resistance is important to ensure effective drugs are used, to prevent the development of extensively drug-resistant TB (XDR-TB), and to prevent onward transmission.

Historically the gold standard drug resistance test has been culture-based phenotypic drug sensitivity testing (DST). However DST for second-line drugs is slow, usually taking weeks, and is not internationally standardized; the results can be poorly reproducible. Several rapid molecular tests, performed directly on clinical samples and giving results within hours, are available to detect resistance. Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) is an automated PCR test that detects rifampicin resistance and was endorsed by the WHO in 2010. The WHO has also endorsed line probe assays (LPAs) including GenoType MTBDRsl (Hain

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Lifescience, Nehren, Germany), which detects several specific resistance mutations for three drug groups including aminoglycosides and fluoroquinolones. However, these rapid molecular tests are limited to testing predetermined drug resistance mutations. The most recent GenoType MTBDRsli v2.0 can test for resistance at 10 different mutation sites, while online databases now include over 1000 drug resistance mutations.

Mycobacterial whole genome sequencing (WGS) can provide a complete genetic resistance profile for all known drug resistance mutations by referencing regularly updated resistance databases. Unlike existing rapid molecular tests, it can differentiate synonymous from non-synonymous mutations by excluding mutations that will not cause an amino acid change. However, WGS generally requires an initial culture stage to ensure enough mycobacterial DNA, as it relies on having sufficient good quality DNA to provide adequate genome coverage depth. This introduces delays that can have significant clinical implications. Mycobacterial DNA from early MGIT culture (Mycobacterial Growth Incubator Tube; BD Diagnostics, Franklin Lakes, NJ, USA) has been used to speed up the time from sample receipt to result to a theoretical 9 days (Pankhurst et al., 2016). WGS directly from unenhanced clinical samples such as sputum has previously yielded low quality DNA that is unsuitable for diagnostics, although this is improved with a human DNA depletion step. A recent study showed that this produced drug resistance information for 24 out of 40 mostly smear-positive samples (Votintseva et al., 2017).

The present authors have previously reported the use of the oligonucleotide enrichment technology SureSelectXT (Agilent, Santa Clara, CA, USA) to obtain Mycobacterium tuberculosis DNA directly from smear-positive and smear-negative sputum samples (Brown et al., 2015), avoiding the need for the initial culture step, to provide WGS data in 20 to 24 smear-positive samples within 96 h. A clinical case where WGS directly from sputum provided a drug resistance profile in a clinically relevant timeframe and altered patient management before alternative testing results were available is reported here.

### Case report

A 29-year-old Nigerian woman presented to the emergency department with a 6-month history of cough and night sweats. She had been diagnosed with presumed pulmonary TB in Nigeria 3 years previously, where she had been treated empirically with rifampicin, isoniazid, pyrazinamide, and ethambutol for 9 months.

Initial induced sputum samples were positive for acid-fast bacilli (AFB) on microscopy. Xpert MTB/RIF, done on all smear-positive samples, confirmed a rpoB mutation highly suggestive of rifampicin resistance and likely MDR-TB. Sputum samples were sent directly to the National Mycobacterial Reference Service (NMRS) for genotypic resistance testing with commercially available kits and to University College London (UCL) for direct enrichment WGS. Sputum samples were inoculated into MGIT and when these flagged positive, mycobacterial isolates were sent to NMRS for first- and second-line phenotypic DST and to UCL for additional WGS. Resistance results and time to identification are shown in Table 1.

Rifampicin resistance was confirmed by Hain LPA (GenoType MDRTBplus v1.0) and WGS on direct samples. The LPA reported a C-15T mutation in the fabG1/inhA promoter, which has a strong association with intermediate isoniazid resistance and ethionamide resistance. WGS testing also identified the inhA S94A mutation, which is also associated with isoniazid and ethionamide resistance, but is not detected by Hain LPAs. Importantly the combination of C-15T and S94A has been reported to be linked to high-level isoniazid resistance (Machado et al., 2013), and this was subsequently confirmed on phenotypic DST. The Hain LPA for second-line drugs (GenoType MDRTBsls v1.0) initially reported a mixed genotype suggestive of fluoroquinolone resistance (Table 1), with weak hybridization of the mutant probe at the gyrA D94G locus.

Based on the LPA results, the patient was initially started on moxifloxacin, amikacin, cycloserine, linezolid, pyrazinamide, ethambutol, and high-dose isoniazid, pending confirmation of

### Table 1

| Resistance patterns identified by Xpert MTB/RIF, GenoType MTBDRplus, GenoType MTBDRsli (performed at NMRS), SureSelectXT WGS, MGIT culture WGS (performed at University College London), and phenotypic testing (performed at NMRS). Mutations identified are stated underneath genotypic resistance results. ‘Days to result’ indicates the number of days for results to be reported to clinicians following receipt in the laboratory. A dash (−) indicates a drug not tested with the assay indicated. |
|---|---|---|---|---|---|
| Xpert MTB/RIF | MTBDRplus v1.0 | MTBDRsli v1.0 | SureSelectXT (WGS) | MGIT culture WGS | Phenotypic |
| Days to result | Rifampicin | Isoniazid | Pyrazinamide | Ethambutol | Moxifloxacin | Ofloxacin | Amikacin | Kanamycin | PAS | Prothionamide | Capreomycin | Linezolid | Streptomycin |
| 0 | Resistant rpoB | – | – | – | Initial: wild-type/resistant gyrA D94G | – | – | – | – | – | – | – |
| 0 | Resistant rpoB H526Y | – | – | – | – | – | – | – | – | – | – | – |
| 1 | Resistant fabG1/inhA C-15T | – | – | – | – | – | – | – | – | – | – | – |
| 5\(^a\) | Resistant fabG1/inhA C-15T | Wild-type | Wild-type/ resistant embB G406D | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type |
| \(^\text{WGS}\) | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type |
| 37 | Resistant rpoB H526Y | – | – | – | Initial: wild-type/resistant gyrA | Repeat: wild-type | – | – | – | – | – | – |
| 29–119\(^b\) | Resistant fabG1/inhA C-15T | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type | Wild-type |

\(^a\) Excluding delays from batching.

\(^b\) Number of days for phenotypic sensitivity to each drug indicated in brackets.

NMRS, National Mycobacterial Reference Service.
fluoroquinolone sensitivity. Direct sputum WGS results provided reassurance that the isolate was sensitive to fluoroquinolones, pyrazinamide, and other drugs used for MDR-TB within 10 days of sample collection (including a 5-day delay due to batching), 12 days before WGS from MGIT samples and 19–109 days before phenotypic DST results. The WHO-approved 9-month MDR-TB regimen was not used given genotypic evidence of high-level isoniazid and ethionamide resistance, the presence of sufficient alternative effective drugs, and to minimize toxicity.

High-dose isoniazid was stopped, as the combination of inhA mutations detected on WGS conferred high resistance, one of which was not tested on LPA. This was an important change, as isoniazid and cycloserine when given concurrently have an increased risk of neurotoxicity. A repeat Hain second-line drug LPA confirmed wild-type gyrA gene.

WGS on the direct sputum sample also reported a mutation consistent with weak ethambutol resistance that was not identified by the LPAs. Repeat WGS on a second sputum sample showed a mixed bacterial population, where 50% of sequencing reads held this mutation and the rest not. This may represent heteroresistance (Eilertson et al., 2014).

A repeat sputum sample sent on day 60 was AFB-negative on smear microscopy and culture. The patient has now completed 18 months of treatment without relapse.

Discussion

The case presented herein demonstrates that WGS can be successfully performed directly from sputum and can identify more second-line drug resistance in a clinically useful timeframe than other currently available methods. While rapid tests such as the Xpert MTB/RIF and LPAs are helpful, the former is limited to mutations in the rpoB gene, while the latter relies on probe hybridization, and they are therefore inherently limited in the number of mutations assessed. Mutations detected at resistance loci not causing an amino acid change and synonymous substitutions have also been incorrectly reported as conferring resistance. WGS is potentially more accurate by allowing scrutiny and interpretation of the exact substitution, as occurred in this case where fluoroquinolone resistance was incorrectly suggested by LPA, and provides a rapid, complete genetic drug resistance profile.

WGS directly from sputum thus promises rapid detection of drug resistance within a clinically relevant timeframe. This could enable early selection of personalized drug treatment, which may be of particular relevance in regions with high rates of second-line drug resistance.

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Ethical approval

The journal’s policy on ethical consent has been read and this work is compliant with it. Samples were collected with informed consent from a TB clinic setting. Approval for the parent study was granted by the NRES Committee East Midlands –Nottingham 1 (REC reference 15/EM/0091). All samples were pseudo-anonymized and allocated a unique identification number.

Conflict of interest

The authors have no conflicts of interest to declare.

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