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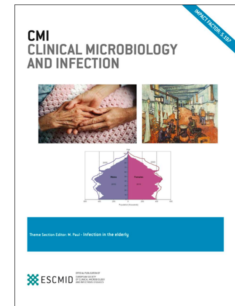

Turnaround time of whole genome sequencing for mycobacterial identification and drug susceptibility testing in routine practice

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PII: S1198-743X(17)30537-2
DOI: 10.1016/j.cmi.2017.10.001
Reference: CMI 1084

To appear in: *Clinical Microbiology and Infection*

Received Date: 9 August 2017
Revised Date: 30 September 2017
Accepted Date: 1 October 2017

Please cite this article as: Olaru ID, Patel H, Kranzer K, Perera N, Turnaround time of whole genome sequencing for mycobacterial identification and drug susceptibility testing in routine practice, *Clinical Microbiology and Infection* (2017), doi: 10.1016/j.cmi.2017.10.001.

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Manuscript type: Research note

Running title: Turnaround time of whole genome sequencing for mycobacteria

Title: Turnaround time of whole genome sequencing for mycobacterial identification and drug susceptibility testing in routine practice

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Keywords: tuberculosis, whole genome sequencing, WGS, mycobacteria

References: 14

Figures: 0; **Tables:** 1.

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Abstract

Objectives: Until recently whole genome sequencing (WGS) for mycobacteria has been restricted mostly to the research setting. However, in 2017 Public Health England has implemented WGS for routine mycobacterial identification and susceptibility testing for *Mycobacterium tuberculosis*. Our objective was to evaluate the impact of this change on the laboratory turnaround times and availability of results.

Methods: Over the years 2016 and 2017, the period January 1st to April 30th was selected to represent before and after implementation of WGS. Prior to 2017, line probe assays were used for mycobacterial species identification. Turnaround times for the different steps of the diagnostic process were evaluated for all positive mycobacterial cultures that were sent from our hospital to the Reference Laboratory during the study period.

Results: A total of 161 positive mycobacterial cultures were sent to the Reference Laboratory. Half of the isolates (n=81/161, 50%) were *M. tuberculosis* and 80/161 (50%) were non-tuberculous mycobacteria. The median number of workdays for mycobacterial species identification was 1 day (interquartile range [IQR] 1-3) in 2016 and 6 days (IQR 5-7) in 2017, $p < 0.001$. For *M. tuberculosis* complex, the median time to drug susceptibility testing results, either molecular or phenotypic, was 12 days (IQR 11-18) in 2016 and 8 days (IQR 7-10) in 2017, $p < 0.001$.

Conclusions: Routine WGS performed well in this setting for mycobacterial identification and susceptibility testing for *M. tuberculosis* and decreased time to drug susceptibility testing results. There was an increase in turnaround times for species identification using WGS, when compared to the previous methods.

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Introduction

Molecular techniques have revolutionized mycobacterial diagnostics over the last decade [1]. Most of the molecular tests currently employed are based on the amplification of a small number of target genes. In contrast, whole genome sequencing (WGS) investigates the whole genome. Technological advances have reduced costs and decreased turnaround time (TAT) of WGS making it more accessible and affordable. In 2017, Public Health England implemented routine-WGS as the primary diagnostic tool for identification of mycobacteria and susceptibility testing for *Mycobacterium tuberculosis* complex (MTBC) [2]. How this change in laboratory practice influenced TAT and availability of results to clinicians is the subject of this report.

Methods

The Clinical Microbiology Department at the Leicester Royal Infirmary is part of the University Hospitals of Leicester NHS Trust with a catchment population of 1 million. Samples submitted for mycobacterial investigations are cultured in liquid (Mycobacterium Growth Indicator Tube (MGIT) system from Becton-Dickinson Microbiology Systems, Cockeysville, MD, USA) and on solid media (Löwenstein Jensen). For mycobacterial blood cultures, the BACTEC (Myco/F-Lytic; Becton Dickinson, Sparks, MD, USA) system is used. Molecular tests using Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) are performed on all new smear positive cases and on clinicians' request. Positive mycobacterial cultures are sent to the National Mycobacteria Reference Service in Birmingham for species identification and drug susceptibility testing for MTBC (DST).

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All positive mycobacterial cultures sent to the National Mycobacteria Reference Service between January 1st – April 30th, 2016 (pre-WGS implementation) and January 1st – April 30th (post-WGS implementation) were included. The TAT, defined as the number of days between receipt of the sample by the reference laboratory and identification of the mycobacterial species, was compared between the two periods. Prior to 2017, mycobacterial species identification was performed using line probe assays (GenoType MTBC and Mycobacterium CM, Hain Lifescience, Nehren, Germany). Phenotypic DST (pDST) for isoniazid, rifampicin, ethambutol and pyrazinamide were performed for all MTBC isolates using the MGIT960 system (Becton Dickinson). Since January 2017, WGS was used for mycobacterial identification. The drug susceptibility profile for MTBC was predicted using WGS data and confirmed by pDST. Whole genome sequencing was performed using the Illumina MiSeq platform (Illumina, San Diego, CA, USA).

Statistical analysis was performed using STATA version 14 (Stata-Corp, TX, USA). The Mann-Whitney U-test was used to evaluate for differences between groups for continuous variables. For categorical variables, the $\chi2$ test was used. The level of significance was set at $\alpha=0.05$.

Results

Sample processing in the primary laboratory

A total of 161 positive cultures, 88/161 (54.7%) MGIT, 72/161 (44.7%) and one BACTEC, were referred to the reference laboratory; 69 in 2016 and 92 in 2017. The majority were respiratory tract samples (n=131/161; 81.4%), followed by lymph node (n=11/161; 6.8%), pleural (n=9/161; 5.6%) and samples from other sites (n=10/161; 6.2%). Median time from sample collection to laboratory receipt was 2 days (IQR 1-3 days); median time to culture positivity was 20 days (12-

30 days). Median time from culture positivity to receipt in the reference laboratory was 2 days (IQR 1-2 days).

Mycobacterial species identification and drug susceptibility testing for MTBC in the reference laboratory

Half of the isolates (n=81/161; 50%) were identified as *M. tuberculosis* complex, 34 in 2016 and 47 in 2017. The remaining 80 were *M. avium-intracellulare* (n=39), *M chelonae/M. abscessus* (n=18) and other non-tuberculous mycobacteria (n=23). Two mycobacterial species could not be identified by WGS. The median number of workdays to mycobacterial species identification was 1 day (IQR 1-3) in 2016 and 6 days (IQR 5-7) in 2017, p <0.001. For MTBC, the median time to DST, either molecular or phenotypic, was 12 days (IQR 11-18) in 2016 and 8 days (IQR 7-10) in 2017, p <0.001. pDST was available after a median 22 days (IQR 20.5-27) in 2017. The median times for various steps of the diagnostic process are shown in Table 1. Delays in WGS results were due to receipt of insufficient sample for 3 samples, failed DNA extraction for 4 samples, poor sequencing data for 3 samples and delays in bioinformatics for 2 samples.

Of 47 confirmed MTBC isolates from 2017, 44 originated from individual patients. On 33 samples, Xpert MTB/RIF was performed on the primary specimen and was positive in 20/33 (60.6%) leading to a more rapid diagnosis.

Phenotypic first-line DST was performed for 46/47 MTBC isolates in 2017. WGS-DST for 45 of these isolates were available, 5 WGS-DST predictions failed and 12 showed mutations of unknown significance for individual first-line drugs. For the remaining isolates WGS-DST were concordant in all isolates for isoniazid, pyrazinamide and ethambutol. There was one discordant result where WGS predicted rifampicin resistance but pDST showed susceptibility.

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Discussion

This study reports the successful implementation of WGS for mycobacterial identification and DST in routine clinical care. Although, TAT for mycobacterial species identification increased from 1 to 6 working days following the implementation of WGS, this is still within the target of 5-7 working days set by Public Health England for WGS [2]. The increased TAT for species identification was offset by the more rapidly available WGS-DST compared to pDST. As with any new method, efficiency is likely to increase once novelty has passed into routine and processes have been optimized. Also, mycobacterial identification by WGS is expected to improve over time with the addition of new database entries. We also found that sample shipment to the reference laboratory and back-reporting of results required an additional 4 days. Therefore, while there is room for improvement of the analytic process itself, tackling delays during the pre- and post-analytic process is equally important and would result in more rapidly available results. The increased use of Xpert MTB/RIF can also lead to a more rapid diagnosis and treatment initiation and compensate for delays [3]. Line probe assays performed directly on primary samples or positive cultures are another means to reduce TAT for DST. However, these were not routinely performed in 2016 due to the low prevalence of drug-resistance in our setting. Due to inconsistent documentation of treatment start dates, we were unable to evaluate if the increase in TAT by using WGS had a clinical impact on time to initiation of anti-tuberculosis therapy. However, other authors report that a considerable proportion of patients, who would subsequently have positive cultures, are started on treatment prior to the availability of the culture result [4].

WGS performed well for species identification for MTBC as well as NTMs and in predicting drug susceptibility for MTBC. There was only one discordance between phenotypic and WGS-

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DST and the number of non-interpretable WGS-DST results were few. The data suggest that WGS-DST has the potential of replacing first-line pDST for settings with a low burden of drug-resistance such as the UK.

WGS has presently a somewhat longer TAT of 3-15 days [5-8], than other already established molecular techniques such as line probe assays [9]. However, data generated by WGS is more comprehensive as it provides additional information beyond species identification and first line drug susceptibility. WGS has higher discriminatory power than the previously used MIRU VNTR typing for investigation of transmission and outbreaks [10]. Furthermore, it allows detection of expected and unexpected laboratory cross-contamination in real-time. For newly diagnosed multidrug-resistant MTBC isolates, WGS is able to predict resistances to second-line drugs [11-13] with the potential for more rapid initiation of appropriate therapy.

Costs for WGS, which were initially prohibitive, have experienced a dramatic decrease now ranging from €150 - 180 per sample processed [6, 7] Moreover, with advancing technologies, there is the potential to use WGS on primary clinical samples [14] which would further reduce TAT of the method.

This study is limited by its retrospective nature, the fact that it is evaluating data from a single center and the lack of drug-resistant isolates reflecting the local tuberculosis epidemiology. Additionally, clinical data were not available and therefore we could not assess how delays in TAT affected clinical outcomes.

In conclusion, routine WGS performed well in this setting for mycobacterial identification and susceptibility testing for MTBC and decreased time to DST results. However, the implementation of WGS resulted in an increased TAT for species identification when compared

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to the previous methods. For MTBC, this could be partially compensated by increasing the use of rapid molecular methods on primary samples.

Transparency declaration

The authors have no conflicts of interest to declare.

Funding

The study had no funding.

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Table 1. Duration in days for the different steps of the specimen processing

Time interval in days, median (IQR)	**Total**	**2016 (LPA)**	**2017 (WGS)**	**p-value**
Sample collection to laboratory registration	2 (1-3)	2 (1-4)	1 (1-3)	p=0.067
Time to culture positivity	20 (12-30)	20 (10-28)	20 (13-32.5)	p=0.409
Time from culture positivity to sending to the reference laboratory	2 (1-2)	2 (2-3)	1 (1-2)	p <0.001
Time to receipt by reference laboratory	1 (1-1)	1 (1-1)	1 (1-1)	p=0.421
Time to identification of mycobacterial species (weekdays)	5 (2-6)	1 (1-3)	6 (5-7)	p <0.001
Time to receipt of result from reference laboratory	1 (1-3)	1 (0-1)	1 (1-3)	p <0.001
Time to phenotypic DST	20 (13-25)	12 (11-18)	22 (20-28)	p <0.001
Time to DST, either phenotypic or molecular	10 (7.5-12.5)	12 (11-18)	8 (7-10)	p <0.001

DST: drug susceptibility testing; LPA: line probe assay; WGS: whole genome sequencing.

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