Institution: University of Oxford		
Unit of Assessment: 1 – Clinical Medicine		
Title of case study: Mycobacterial sequence-based diagnostics improve tuberculosis services		
Period when the underpinning research was undertaken: Jan 2010 - Dec 2019		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Timothy Walker	*PHE Clinical Research Fellow;* Clinical Lecturer, Wellcome Trust Career Development Fellow	*2010 – 2012 (Category C, Public Health England);* Sep 2015 – present
Derrick Crook	*Consultant in Infectious Diseases and Clinical Microbiology*	*1996 – present (Category C, OUH NHS)*
Tim Peto	*Consultant Physician*	*1988 – present (Category C, OUH NHS)*
(Ann) Sarah Walker	Professor of Medical Statistics and Epidemiology	Dec 2012– present
Zamin Iqbal	Group Leader; Sir Henry Dale Fellow	Jul 2009 – Dec 2016
Philip Fowler	University Research Lecturer	Feb 2006 – present
Period when the claimed impact occurred: Aug 2013 – Dec 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

University of Oxford researchers have developed, from concept to implementation, a whole genome sequencing (WGS)-based mycobacterial diagnostic solution, jointly with Public Health England. Implemented in January 2018, it is a fully accredited national service and is the first end-to-end solution in the world, replacing routine culture-based reference and clinical diagnostic services with a cheaper, faster and higher quality service for tuberculosis (TB). The University of Oxford team also developed the tools and demonstrated the efficacy of WGS for predicting drug resistance for *Mycobacterium tuberculosis*. These approaches have been adopted into routine public health practice internationally, including in the US, EU, and Australia, achieving faster, more accurate and cost-effective diagnostics. Clinicians and patients have benefitted from faster confirmation of appropriate drug treatments, and there has been improved precision in public health interventions to prevent transmission.

2. Underpinning research

Crook, Peto, A.S. Walker, T. Walker and colleagues at the University of Oxford developed a suite of WGS-based tools for mycobacterial diagnostics. Their research showed the validity, accuracy, and utility of WGS-based analysis of Mycobacteria, particularly *M. tuberculosis*, which causes TB, allowing these tools to replace previous cumbersome, slow or error-prone diagnostic methods.

Optimised Mycobacteria DNA extraction and WGS library preparation culture.
The standard approach for growing TB is by Mycobacteria Growth Indicator Tube (MGIT) liquid culture, in which automated detection of growth occurs at a low mycobacterial load. To enable WGS, Crook and colleagues developed and validated an optimal method for DNA extraction from MGIT tubes as soon as growth is detected, yielding sufficient DNA for WGS library preparation and sequencing on Illumina platforms [1].

Diagnosing species and drug susceptibility
Key information needed by clinicians to treat a patient with suspected TB is whether the bacterial infection is *M.tuberculosis* or another Mycobacterium, and whether it is resistant or susceptible to specific antibiotics. University of Oxford researchers, including Crook, Peto and Iqbal, developed software tools and catalogues of sequence variants to provide this information from WGS of a mycobacterial isolate. By sequencing the genomes of all described *Mycobacterium* species, they constructed tools, including MyKrobe Predictor [2], for species identification.

The University of Oxford researchers assembled large catalogues relating mycobacterial DNA sequence variants to drug resistance, both for first- and second-line antibiotics used to treat TB [3, 4]. Their initial work [3] quantified the association of genomic variation and anti-tuberculosis drug resistance in more than 3,500 whole genome-sequenced isolates, demonstrating their method had high sensitivity and specificity for predicting resistance. Following this success, they led an international consortium called CRyPTIC (Comprehensive Resistance Prediction for Tuberculosis: an International Consortium) to analyse more than 10,000 strains, demonstrating that their sequence-based prediction method accurately identified at least 97% of drug-susceptible isolates, for the four first-line drugs (isoniazid, rifampicin, ethambutol and pyrazinamide) [4]. This showed for the first time that understanding the genome sequence is accurate enough to confirm whether these drugs will be effective in the clinic, and showed that WGS-based susceptibility testing could replace phenotypic (culture-based) testing.

Accurate identification of transmission clusters by sequence analysis

Identifying relatedness between TB isolates is essential to trace transmission and guide public health interventions. The standard mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) method could suggest clusters of isolates, but lacked sufficient resolution to be certain of transmission events. University of Oxford researchers looked at single nucleotide polymorphisms (SNPs) from WGS and computed a molecular clock from genomically- and epidemiologically-related samples. This approach produced highly accurate genetic distances and outbreak identification, with far greater resolution than previous methods, providing a robust, improved framework for identifying transmission clusters [5].

Demonstrating WGS-based tools for mycobacterial diagnosis

Using their bioinformatics tools, incorporated into automated software (COMPASS), and WGS-analysis methods, Crook and University of Oxford researchers collaborated with clinical and public health laboratories to compare their approaches head-to-head with routine diagnostic workflows. They showed that sequencing could be cost efficient and improve turn-around-time and accuracy of diagnostics [6].

3. References to the research (University of Oxford employees in bold, students in italic)

1. **Votintseva AA**, **Pankhurst LJ, Anson LW, Morgan MR**, Gascoyne-Binzi D, *Walker TM*, **Quan TP, Wyllie DH, Del Ojo Elias C,** Wilcox M, **Walker AS, Peto TE, Crook DW** (2015). Mycobacterial DNA extraction for whole-genome sequencing from early positive liquid (MGIT) cultures. *J Clin Microbiol.* 53:1137-43. doi:10.1128/JCM.03073-14. Citations: 61 (Google Scholar, 02-2021)
2. **Bradley P**…**Walker AS, Peto TEA, Crook DW, Iqbal Z** (2015). *[22/29 authors at University of Oxford].* Rapid antibiotic-resistance predictions from genome sequence data for Staphylococcus aureus and Mycobacterium tuberculosis. *Nat Commun.* 6:10063. doi: 10.1038/ncomms10063 Citations: 365 (Google Scholar, 02-2021)
3. *Walker TM,* Kohl TA, Omar SV, **Hedge J**…**Peto TEA** (2015) *[13/26 named authors at University of Oxford].* Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. *Lancet Infect Dis.* 15:1193-202. doi:10.1016/S1473-3099(15)00062-6. Citations: 415 (Google Scholar, 02-2021)
4. CRyPTIC Consortium (2018). *[15/92 authors at University of Oxford;* **Walker TM, Walker AS, Peto TEA** *as writing group]*. Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing. *N Engl J Med.* 379:1403-15. doi:10.1056/NEJMoa1800474. Citations: 134 (WoS 03-2021)
5. **Walker TM, Ip CL,** Harrell RH,..**Peto TEA** (2013). *[8/17 authors at University of Oxford].* Whole-genome sequencing to delineate Mycobacterium tuberculosis outbreaks: a retrospective observational study. *Lancet Infect Dis.* 2013;13:137-46. doi:10.1016/S1473-3099(12)70277-3. Citations: 680 (Google Scholar, 02-2021)
6. **Pankhurst LJ, del Ojo Elias C, Votintseva A, Walker TM**…**Crook DW** (2016). *[9/23 named authors at University of Oxford].* Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study. *Lancet Respir Med.* 4:49-58. doi:10.1016/S2213-2600(15)00466-X Citations: 253 (Google Scholar, 02-2021)

Funding to Crook at the University of Oxford includes Gates Foundation (USD1,013,923 for CRyPTIC); Wellcome Trust (087646/Z/08/Z, GBP2,829,138); MRC (GBP2,588,140, G0800778).

4. Details of the impact

TB is serious respiratory infection caused by *M. tuberculosis*, and is the leading cause of death from a bacterial infection. In 2019, there were approximately 10,000,000 cases of TB and 1,400,000 fatalities, and tackling TB is a priority of the World Health Organisation (WHO). A major problem is the rapid rise in drug-resistant strains; the WHO estimates that in 2018 there were 484,000 new cases with resistance to the most effective first-line drug (rifampicin), 78% of which had multidrug resistance. Clinicians require accurate diagnosis of whether an infection is *M. tuberculosis* or another Mycobacterium, and whether the infection is susceptible or resistant to specific antibiotics. Public health bodies need to be able to identify the relatedness of different TB cases to act to control transmission. The University of Oxford research showed that WGS can yield all this information, and provided the evidence and tools that have enabled improved comprehensive mycobacterial diagnostics at national and international levels.

World's first implementation of large-scale WGS-based disease diagnostics in England

England has one of the highest rates of TB in western Europe. Public Health England (PHE) and the University of Oxford collaborated to develop, validate and implement an end-to-end mycobacterial diagnostic service, from the clinical sample through to the results report [6]. Implementation of this service [A] was completed in Jan 2018 and achieved full UKAS accreditation in late 2019. As announced by the UK government in March 2017, this was the first use of WGS in diagnostics for any disease at this scale [B]. The PHE pipeline incorporates University of Oxford approaches for DNA extraction [1], and analysis of sequencing data using the University of Oxford-developed COMPASS pipeline [6] to identify species and drug susceptibility [2, 3, 4], and relatedness [5] [A]. With phased introduction starting in Dec 2016, since Jan 2018 all positive mycobacterial cultures in England have been analysed through this WGS pipeline at reference laboratories in London and Birmingham [A]. In 2019, a total of 14,605 mycobacterial cultures were processed for WGS by PHE. Based on University of Oxford-led research [4], since 2018 phenotypic susceptibility testing for *M. tuberculosis* was stopped for 80% of isolates [A].

International adoption of mycobacterial WGS

WGS of most or all mycobacterial samples has been implemented by several public health bodies including in the USA, Netherlands, Italy, Australia, Germany and Canada. The University of Oxford research, and pipeline adopted by PHE, influenced these decisions and the approaches used.

New York State: The New York State Department of Health, Wadsworth Center, have been developing their pipeline for mycobacterial WGS with input from University of Oxford researchers since 2015 [C]. This includes adopting laboratory assays [1], which contributed to the successful Wadsworth application for regulatory approval, and incorporating methods into an analysis algorithm [C]. These WGS approaches became routine from Oct 2018 and enabled replacement of phenotypic drug susceptibility testing for 80% of *M. tuberculosis* isolates [C]. Since 2016, Wadsworth have used WGS for 3500 TB isolates, with approximately 800 TB cases identified per year [C]. Wadsworth shared the pipeline with other US states and the US Association of Public Health Laboratories, to provide a centralised platform [C].

Netherlands: In the Netherlands, WGS is now used for all *M. tuberculosis* complex isolates (approximately 600 per year) and, on the basis of University of Oxford research [4], phenotypic resistance testing has been discontinued for the 90% of isolates that have no resistance mutations in WGS [D]. According to the Head of the Netherlands Tuberculosis Reference Laboratory at the National Institute for Public Health and the Environment, "*The impact of the research at the Oxford University regarding the introduction of WGS is major…This formed the basis to a true revolution in the diagnosis of this disease"* [D].

Italy, Australia, and others: The Italian supranational reference laboratory for TB now offers routine WGS for patients at high risk of drug-resistant TB. The head of this laboratory stated that "*The Oxford University research contribution was a major breakthrough in support for the implementation of WGS*" and that interpretation of TB WGS results in Italy is based on evidence from University of Oxford research [E]. Based directly on input from Crook, T. Walker and colleagues, the lead of the Mycobacterium Reference Laboratory in New South Wales (NSW), Australia, established WGS for all clinical isolates (over 1,200 isolate) of *M. tuberculosis* (piloted

from Oct 2016, routine service from July 2019) [F]. The University of Oxford research [e.g. 4] was critical for the design of the genomics service, and has resulted in more accurate and rapid detection of drug-resistant TB and improvement in the resolution and timeliness of detection of clusters of recent community transmission [F]. The NSW implementation led to roll-out of similar programmes in other Australian states in 2019 [F]. Routine WGS for TB is also now used in other countries including Germany and Canada.

World Health Organisation: Based on their research on sequencing-based drug susceptibility prediction [4], the University of Oxford team was contracted by the Foundation for Innovative Diagnostics (FIND) to provide a catalogue of genetic variants associated with drug susceptibility for the WHO [Gi,ii]. In Dec 2020, the WHO confirmed that the catalogue, based on WGS of 40,000 isolates, will form the basis of a new reference standard for national and international TB programmes and industry [Gi,ii].

Benefits of routine mycobacterial WGS

The implementation of WGS for Mycobacteria has led to benefits for public health laboratories, clinical care for patients, and in public health responses.

Decreased turnaround times, costs, and risks in public health laboratories: Because WGS yields all the required diagnostic and epidemiological information, the reports are available faster than by separate conventional methods. For example: PHE report drug susceptibility within 5-7 days, compared to 3-4 weeks previously [A]; and Wadsworth have a 7-day average turnaround time for reporting susceptibility, which is 7 days earlier than previously for first-line drugs and several weeks faster for second-line drugs [C].

Although WGS is an expensive technology, the end-to-end pipeline removes the need for numerous laboratory assays, and PHE estimated saving approximately 7% per sample by using WGS [6]. The Netherlands implementation has been "*highly cost effective*" because of the reduced need for other expensive and labour-intensive methods [D], and Wadsworth report "*savings in staff time and cost savings*", which are covering the costs of implementing WGS and freeing up staff to develop further testing improvements [C]. The reduction in the number of laboratory assays enabled by WGS also improves safety as technicians are less frequently exposed to the hazards of working with viable bacteria in biosafety level 3 laboratories, reducing the risk of laboratory infections [D].

Faster, tailored clinical care: The rapid return to clinicians of drug susceptibility results from WGS has decreased the time to treat patients with appropriate antibiotics. For cases where the isolate is shown to be susceptible to first-line drugs, clinicians are given rapid reassurance that they are using the appropriate regimen [A, C, D]. Crucially, patients with drug-resistant infections previously had to wait several weeks for effective treatment and they now receive it in 7 days, transforming their clinical care. For example, in 2017-2018 in England, an effective treatment regime was identified 3-16 weeks earlier for 700 patients with drug-resistant TB [A]. When TB is treated in hospital, it is expensive to healthcare providers – for example, approximately GBP900 per day in an airflow-controlled ward – and rapid identification of an effective treatment regime speeds up discharge, yielding further savings. Choice of drugs for a patient is also now personalized when the WGS shows identical resistance mutations to an infection in a previously treated patient [A]. Additionally, WGS reveals low-level resistance to the first-line drug rifampicin, which was previously very difficult to detect, so clinicians are now able to tailor drug treatment for patients infected with these strains [C, D].

Improved precision in public health interventions: According to PHE, the speed and increased resolution of SNP typing has transformed cluster investigation to prevent further cases [A]. For example, PHE has been able to: pinpoint transmission between specific individuals, avoiding unnecessary extensive contact tracing efforts; focus a targeted public health intervention in a healthcare setting where rapid transmission through brief contact was discovered to have occurred; identify and address transmission due to breaches in infection control practice in a healthcare setting, avoiding further risk to patients; and contribute to stopping the spread of international clusters of extensively drug resistant TB [A].

WGS in routine use for UK bovine TB surveillance

Bovine TB, caused by *Mycobacterium bovis*, is a major problem in the UK, leading to compulsory slaughter of approximately 50,000 cattle annually and costs of approximately GBP150,000,000 to UK taxpayers in eradication efforts, plus additional costs for the cattle industry. A 2018 government

review determined it is "*feasible and cost-efficient to move to whole-genome sequencing…* [which] *allows disease transmission pathways to be identified with greater accuracy*" [Hi]. This has been enabled by the UK Animal and Plant Health Agency (APHA) adopting a modified version of the University of Oxford-PHE human TB pipeline in 2018. APHA has validated and accredited WGS, and since 2019 it is in routine use for surveillance and research into the control of TB [Hii]. APHA uses a web portal called 'ViewBovine' developed with Peto and University of Oxford colleagues to utilize the WGS data [Hii]. These University of Oxford tools have been used by APHA to identify infection pathways and sources more rapidly, accurately and with greater certainty than standard methods [I].

Motivating public interest in medical research through citizen science

Bash the Bug (set up by Fowler and University of Oxford colleagues in April 2017) is the most popular biomedical citizen science project on the Zooniverse platform. Volunteers help to classify images of bacteria growing on a microtitre plate developed by the CRyPTIC consortium to gather data on the drug susceptibility of approximately 20,000 *M. tuberculosis* samples, which are subject to WGS. More than 45,000 users from at least 19 countries have contributed more than 4,500,000 image classifications up to August 2020. A survey of Zooniverse volunteers by the University of Nottingham (May 2020) found that 83% of 92 respondents who listed Bash the Bug as their favourite project had sought out ways to learn about scientific topics since taking part [Ji]. Similarly, in response to a single-question online poll (Oct 2020), 43 of 49 respondents (88%) agreed with the statement "*BashtheBug has increased my interest in medical research*" [Jii]. Comments received from volunteers showed Bash the Bug has provided benefits to some people in empowering them to help medical research, particularly during the COVID-19 pandemic, when notably there was a huge spike in classifications in April-May 2020 [Jii].

5. Sources to corroborate the impact

A. Letter from Clinical Lead for Public Health England Mycobacterial Reference Service for the Midlands (Oct 2020), confirming role in the PHE TB pipeline and impacts of implementation.

B. UK government press release announcing PHE implementation of WGS for TB, including the University of Oxford's role.

C. Letter from Chief of Bacterial Diseases, Wadsworth Center, US, (Oct 2020), detailing their implementation of mycobacterial WGS, the influence of University of Oxford research, and impacts of implementation.

D. Letter from Head of the Netherlands Tuberculosis Reference Laboratory at the National Institute for Public Health and the Environment (Oct 2020) detailing implementation of mycobacterial WGS, the influence of the research, and impacts of implementation.

E. Letter from Head of TB Supranational Reference Laboratory, Italy (Nov 2020), stating contribution of University of Oxford research to implementation of WGS.

F. Letter from lead at Centre for Infectious Diseases & Microbiology Laboratory Services, NSW, Australia (Nov 2020), detailing contribution of University of Oxford work to implementation of WGS for TB, its benefits for TB diagnostics, public health and subsequent influence on broader Australian implementation and policies.

G. Letters describing role of University of Oxford in *M. tuberculosis* mutation catalogue (Dec 2020) from: [i] Team Leader, Global TB Programme, WHO; [ii] Head of Sequencing, FIND.

H. UK government reports on bovine TB: [i] Government bovine TB strategy report 2018, stating feasibility of WGS, p4; [ii] UK Animal and Plant Health Agency Annual Report 2018 stating adoption of WGS and University of Oxford tools, p21, 61; and 2019 referring to routine use of WGS in surveillance and research, p35.

I. Yang-Turner F, Volk D, Roberts T, Herreros J, Ellis R, Peto T (2020). ViewBovine: A Microservices-powered Web Application to Support Interactive Investigation of Bovine Tuberculosis Infection Pathways. IEEE World Congress on Services, pp. 4-6, doi:10.1109/SERVICES48979.2020.00014. Describing use of ViewBovine by APHA.

J. Anonymous data from surveying Bash the Bug volunteers: [i] Data from survey of Zooniverse volunteers, May 2020, provided by the University of Nottingham; [ii] Responses and comments from a one-question poll and opportunity to comment (P. Fowler, Oct 2020).