Added value of whole-genome sequencing for management of highly drug-resistant TB

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Objectives: Phenotypic drug susceptibility testing (DST) for *Mycobacterium tuberculosis* takes several weeks to complete and second-line DST is often poorly reproducible, potentially leading to compromised clinical decisions. Following a fatal case of XDR TB, we investigated the potential benefit of using whole-genome sequencing to generate an *in silico* drug susceptibility profile.

Methods: The clinical course of the patient was reviewed, assessing the times at which phenotypic DST data became available and changes made to the therapeutic regimen. Whole-genome sequencing was performed on the earliest available isolate and variants associated with drug resistance were identified.

Results: The final DST report, including second-line drugs, was issued 10 weeks after patient presentation and 8 weeks after initial growth of *M. tuberculosis*. In the interim, the patient may have received a compromised regimen that had the potential to select for further drug resistance. The *in silico* susceptibility profile, extrapolated from evolving evidence in the literature, provided comparable or superior data to the DST results for second-line drugs and could be generated in a much shorter timeframe.

Conclusions: We propose routine whole-genome sequencing of all MDR *M. tuberculosis* isolates in adequately resourced settings. This will improve individual patient care, monitor for transmission events and advance our understanding of resistance-associated mutations.

Keywords: extensively drug resistant, multidrug resistant, XDR, MDR

Introduction

The global TB epidemic is increasingly being driven by the emergence and spread of drug-resistant strains.[1] The WHO estimates that nearly half a million (450 000; range: 300 000–600 000) people developed MDR TB (resistant to isoniazid and rifampicin) in 2012, of whom <20% received appropriate treatment.[2] The diagnosis and treatment of MDR TB present major challenges. Dependence on traditional drug susceptibility testing (DST) incurs significant delays in diagnosis, which force clinicians to initiate empirical treatment that may be suboptimal, leading to the multiplication of drug resistance and adverse patient outcomes.

Initiating treatment with an optimal regimen is especially important in patients with XDR TB (MDR with additional resistance to fluoroquinolones and at least one second-line injectable drug), since therapeutic options remain limited. Approximately 10% of MDR cases are thought to be XDR[2] and its global rise is driven by treatment that is poorly aligned with local resistance patterns,[3] as well as by efficient transmission of some XDR strains.[4] Given the poor treatment outcomes of XDR TB patients,[5] every effort should be made to reduce delays in case detection and characterization of susceptibility profiles.

Conventional phenotypic DST requires a minimum of 2 weeks: one for initial detection of microbial growth and another to assess

critical concentrations of first-line anti-TB drugs.[6] In reality, full characterization of an XDR strain may take months, since expanded DST is usually performed sequentially and often some tests need to be repeated. In addition, drug resistance breakpoints for most second-line drugs are poorly standardized. Genotypic tests such as the commercial GeneXpert MTB/RIF® and GenoType MTBDRplus® (Hain Lifescience) assays offer rapid assessment of drug resistance mutations against key first-line anti-TB drugs, with testing of second-line agents using the GenoType MTBDRsl® (Hain Lifescience) assay if required. However, culture-based DST remains the reference standard, given the limited number of mutations evaluated by current genotypic tests and our incomplete understanding of the mechanisms of resistance.

Whole-genome sequencing has offered novel insight into the evolution and spread of *Mycobacterium tuberculosis*, but its application in routine patient care has been limited by the perceived lack of clinical relevance, high cost, complexity of interpretation and slow turn-around times. Recent technological advances have made routine whole-genome sequencing of select *M. tuberculosis* strains in reference laboratories in a clinically relevant timeframe technically and economically feasible. We present a case of XDR TB diagnosed in New South Wales, Australia, and illustrate the potential clinical value of routine whole-genome sequencing of highly resistant *M. tuberculosis* strains.

Patient and methods

A man in his thirties presented to hospital with cough, night sweats and weight loss for more than 3 months. He was of East African ancestry and had spent much of his adult life as a refugee in the Horn of Africa. He first received treatment for TB in Ethiopia in early adolescence; the basis for the diagnosis was uncertain and he discontinued treatment after 3 months due to war. A decade later in Djibouti, he was treated for sputum smear-positive TB. He received 6 months of standard first-line therapy, comprising isoniazid, rifampicin, pyrazinamide and ethambutol during the 2 month intensive phase, with isoniazid/rifampicin during the 4 month continuation phase. He was documented to be sputum smear negative after completion and declared cured.

Three years later he was diagnosed with a third episode of TB. His third treatment course (also received in Djibouti) comprised 2 months of isoniazid/rifampicin/pyrazinamide/ethambutol and streptomycin, followed by 1 month of isoniazid/rifampicin/pyrazinamide/ethambutol and a further 5 months of isoniazid/rifampicin/ethambutol. He was reported to be culture negative at the end of treatment. He subsequently received approval to settle in Australia, on condition that he commit to regular TB screening. He had stable chest radiograph findings and culture-negative sputum for 2 years after migration (Figure 1a), but was subsequently lost to follow-up until his symptomatic presentation, 5 years after migration.

Physical examination on presentation revealed cachexia (38 kg) and finger clubbing. The chest radiograph showed bilateral cavities with extensive fibrocalcific changes (Figure 1b). Sputum microscopy was 3+ for acid-fast bacilli and *M. tuberculosis* was confirmed on culture. HIV serology was negative. Given his long disease-free interval and poor clinical condition, the patient was commenced immediately on regular first-line therapy with daily isoniazid/rifampicin/pyrazinamide/ethambutol, pending the outcome of genotypic and phenotypic DST. GeneXpert MTB/RIF® testing was not performed, but GenoType MTBDRplus® (Hain Lifescience) assay results suggested MDR TB. When this information became available at week 3, amikacin and moxifloxacin were added to his regimen.

Five weeks after presentation, phenotypic DST results showed resistance to isoniazid (MIC >0.4 mg/L), rifampicin and pyrazinamide, with ethambutol susceptibility. Additional results suggested resistance to
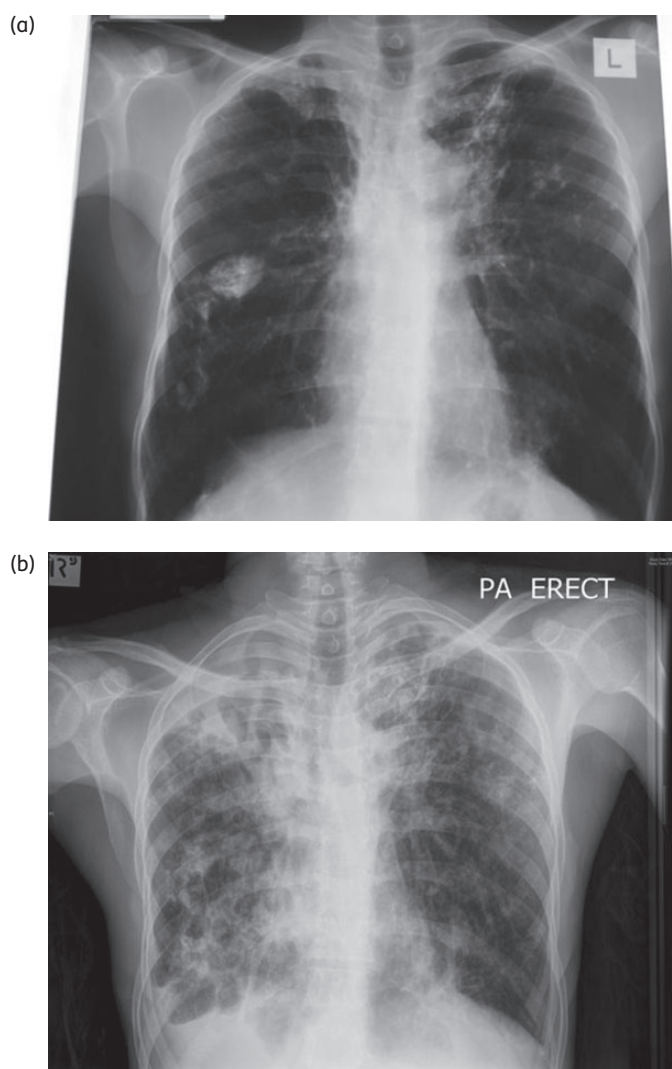

Figure 1. (a) Chest radiograph when asymptomatic; 3 years before presentation. (b) Chest radiograph with symptomatic presentation.

streptomycin, amikacin, capreomycin, ciprofloxacin and ethionamide, fulfilling the WHO definition of XDR TB. All medications were temporarily ceased until more complete second-line susceptibilities became available. The expanded DST report, issued 10 weeks after initial diagnosis, listed susceptibility to *para*-aminosalicylic acid, cycloserine and clofazimine. He was restarted on moxifloxacin, high-dose ethambutol (25 mg/kg) and pyrazinamide with the addition of *para*-aminosalicylic acid, cycloserine, clofazimine and amoxicillin/clavulanate. On this regimen his clinical situation improved, with no further fevers, reduction in cough, 17 kg of weight gain and some radiological improvement. Unfortunately the patient developed major depression and cycloserine had to be discontinued; it was replaced by trimethoprim/sulfamethoxazole and clarithromycin. Susceptibility testing on an isolate collected 9 months after presentation reported intermediate-level resistance to moxifloxacin (MIC between 1.0 and 2.0 mg/L) and susceptibility to linezolid.

The patient remained in hospital in strict isolation in a negative-pressure room for 2 years, since his sputum remained culture positive for *M. tuberculosis* despite smear conversion. Given his stable clinical condition, suitable accommodation was ultimately found where the infectious risk could be minimized and directly observed therapy provided 7 days a week. However, within 4 months of discharge he was readmitted

to hospital with extensive ascites, he was culture positive for *M. tuberculosis* and he died following a major pulmonary haemorrhage.

Whole-genome sequencing

After the patient's death, an early *M. tuberculosis* isolate was submitted for whole-genome sequencing using the Illumina HiSeq 2000 platform, producing 100 base paired-end reads with >500-fold average depth of coverage after mapping onto the *M. tuberculosis* H37Rv reference genome (NCBI GenBank NC_000962).

Several genetic variants in different genes associated with drug resistance in *M. tuberculosis* were identified by whole-genome sequencing, providing a plausible genetic basis for the observed phenotypic resistance to isoniazid, rifampicin and pyrazinamide, as well as all fluoroquinolones and injectables (capreomycin and aminoglycosides).[7–11] These are shown in

Table 1. Although this patient's strain was initially reported to be susceptible to ethambutol using phenotypic methods, the sequenced isolate possessed multiple mutations associated with ethambutol resistance.

Discussion

In retrospect, this patient's treatment might have been optimized to provide him with the best possible chance of cure if whole-genome sequencing data had been available at the time. More importantly, in future the growing body of knowledge on molecular markers of drug resistance and the availability of new antimycobacterial agents should provide better guidance and more treatment options to clinicians. In our case, the sequenced isolate had genetic evidence of resistance to ethambutol, pyrazinamide

Table 1. Genetic variants[a] identified with potential to contribute to drug resistance

Drug	Gene	H37Rv identifier	Variant	Likelihood of resistance[b]	Clinical relevance
Isoniazid[7,11]	*katG*	Rv1908c	S315T	high	no value in treatment
	fabD	Rv2243	S275N	high	
	iniA	Rv0342	H481Q	low	
	Rv1592c	Rv1592c	I322V	low	
	proA	Rv2427c	V140L	low	
Rifampicin[7,11]	*rpoB*	Rv0667	S450W[c]	high	no value in treatment
Pyrazinamide[7,11]	*pncA*	Rv2043c	D158EAG[d]	high	no value in treatment
			T160M	high	
			T167A	low	
Ethambutol[7,11]	*embC*	Rv3793	T270I	possible	no value in treatment
			N394D	possible	
	embA	Rv3794	V206M	possible	
			P913S	possible	
	embB	Rv3795	D328G	possible	
			E378A	possible	
			G406S	high	
	rmlD	Rv3266c	S257P	possible	
	embR	Rv1267c	D107N	possible	
			C110Y	possible	
	iniA	Rv0342	H481Q	possible	
Fluoroquinolones[7–9,11]	*gyrA*	Rv0006	E21Q	low	limited value, should not be counted as an 'active drug'
			A90V[e]	high	
			D94Y[e]	high	
			A384V	very low	
			G668D	very low[26]	
	gyrB	Rv0005	M330I	very low	
			E540D	low[27]	
Aminoglycosides, streptomycin and capreomycin[7,10]	*rrs*	Rvnr01	A1401G	high	no value in treatment
	rpsL	Rv0682	K43R	high	
	gidB	Rv3919c	S100F	possible	
Ethionamide[7]	*ethA*	Rv3854c	C403W	possible	limited value, should not be counted as an 'active drug'

[a]Compared with H37Rv as the reference strain.
[b]High—strong evidence for a causal association with a drug-resistant phenotype; low—conflicting evidence or evidence suggesting a weak association with a drug-resistant phenotype; possible—theoretical possibility of causal association with a drug-resistant phenotype, but insufficient evidence.
[c]S531W with *Escherichia coli* numbering.
[d]Frameshift mutation.
[e]Heteroresistance—mutations detected in 63% (A90V) and 16% (D94Y) of reads.

and moxifloxacin, which were components of both his expanded first-line regimen and his initial XDR regimen. When XDR TB treatment was ultimately commenced, the only drugs in the regimen with likely activity were *para*-aminosalicylic acid and cycloserine, with possible contributions from clofazimine and amoxicillin/clavulanate.

The regimen was suboptimal due to delayed DST results and inadequate laboratory guidance on likely ethambutol resistance. The difficulties of establishing ethambutol breakpoints and performing reliable phenotypic DST have been documented before.[12] In addition, DST results arrived in stages over many weeks, hampering rational treatment decision-making. This is of particular importance in TB, where three to four active drugs are required to prevent amplification of drug resistance, and the addition of a single drug to a failing regimen is irresponsible. The therapeutic regimen was compromised when cycloserine had to be ceased and was replaced with trimethoprim/sulfamethoxazole, which act on the same metabolic pathway as *para*-aminosalicylic acid, and clarithromycin, which has little activity against *M. tuberculosis*.[13,14] With the availability of genomic information, the objective of including at least four active drugs[15] might have been achieved if the addition of a carbapenem[16] or linezolid[17] was considered early in the treatment course. Linezolid was not included in the regimen because of its significant side effect profile, minimal efficacy data and the belief that four active agents were available (ethambutol, PAS, cycloserine and clofazimine). The patient did show clinical improvement on this regimen. Neither delamanid[18] nor bedaquiline[19] was available and there was little evidence at that time to suggest that carbapenems were sufficiently effective to warrant inclusion.

This case study emphasizes the heavy clinical reliance on phenotypic DST, which is too slow and insufficiently validated for drugs other than isoniazid, rifampicin, quinolones and the injectables to guide individual patient management. Our patient's treatment failed despite the anticipation of MDR TB and the prompt addition of two extra drugs (moxifloxacin and amikacin) to his regimen following preliminary genetic testing for MDR. Given the urgency to optimize treatment before additional drug resistance develops, every effort should be made to optimize the regimen from the very beginning.

We propose that all isolates found to be likely MDR on initial genetic testing (GeneXpert MTB/RIF® or GenoType MTBDRplus®) should undergo expedited whole-genome sequencing. A realistic turn-around time for whole-genome sequencing is 1–2 weeks from nucleic acid extraction and costs of US$70–250 per isolate pale in comparison with patient management costs. The estimated total cost of this patient's care was equivalent to US$1 million, with average XDR TB treatment costs in developed countries estimated at US$483 000.[20] The price and turn-around time of whole-genome sequencing is rapidly decreasing with ongoing technological advances. Routine whole-genome sequencing would also facilitate detailed transmission analysis, to guide targeted public health interventions and monitor infection control practices.[21–25] Phenotypic testing would still be required to validate findings, comply with international recommendations and assist the elucidation of unknown resistance mechanisms, but this may change in future with growing confidence in the genetic markers of resistance. An additional safety advantage of whole-genome sequencing for laboratory personnel can be obtained if there are fewer manipulations of live cultures.

Routine whole-genome sequencing of all MDR *M. tuberculosis* isolates will assist patient management and guide public health responses, but is currently feasible only in well-resourced settings. It is important to drive innovation and refine the clinical application of this new technology, since widespread use is anticipated in the near future and TB patients globally stand to benefit.

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Transparency declarations

None to declare.

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