Transmitted Extended-Spectrum Extensively Drug-Resistant Tuberculosis in Beijing, China, with Discordant Whole-Genome Sequencing Analysis Results

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Drug resistance to tuberculosis remains a major public health threat. Here, we report two cases of extended-spectrum extensively drug-resistant (XXDR) tuberculosis showing resistance to most first- and second-line agents. The results of a correlation of whole-genome sequencing (WGS) and phenotypic testing were discordant, suggesting that overreliance on WGS may miss clinically relevant resistance in extensively drug-resistant disease.

Patient EF, a 64-year-old HIV-negative Han Chinese male, was diagnosed with sputum culture-positive tuberculosis (TB) at a hospital in 2011. Comorbidities included type II diabetes and chronic hepatitis C infection. Initial drug susceptibility testing (DST) results suggested that the tuberculosis was caused by a strain of *Mycobacterium tuberculosis* that was resistant to rifampin, isoniazid, ethambutol, and streptomycin; therefore, treatment with levofloxacin, para-aminosalicylate (PAS), and amikacin was initiated. Over the course of the next 45 days, the patient continued to lose weight (loss of 10 kg), and the patient was transferred to the Beijing Chest Hospital, where analysis using an extended DST panel was performed on a newly isolated strain (strain 8897) (Table 1). In the meantime, his treatment regimen was changed to rifapentine, high-dose isoniazid, ethambutol, pyrazinamide, and levofloxacin. The patient remained sputum culture positive and subsequently succumbed to newly diagnosed hepatic cancer in January 2013.

Patient ZX, a 30-year-old man, was initially diagnosed with sputum smear-positive tuberculosis in 2010 at a hospital after presenting with a cough and night sweats. He was treated with standard therapy (rifampin, isoniazid, pyrazinamide, and ethambutol), with initially good outcome, although his cough persisted. After 6 months, the patient was still sputum culture positive, and DST indicated that the *M. tuberculosis* strain was resistant to rifampin, isoniazid, streptomycin, capreomycin, kanamycin, amikacin, and ofloxacin but remained susceptible to ethambutol, prothionamide, PAS, and levofloxacin. The patient's treatment was therefore changed to pyrazinamide, ethambutol, and prothionamide, and treatment with 3 months of PAS was added. By September 2012, further DST revealed novel resistance to PAS and prothionamide, but the strain was now sensitive to ethambutol, levofloxacin, moxifloxacin, and capreomycin. Computed tomography (CT) imaging of the chest suggested that an increase in disease burden had occurred; therefore, he was transferred to the Beijing Chest Hospital and a tuberculosis strain (strain 11500) newly isolated from him was tested for drug sensitivities. The patient was not diabetic and tested negative for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and HCV. His antibiotic regimen was changed to pyrazinamide, ethambutol, clofazimine, moxifloxacin, linezolid, and

capreomycin. Due to an inadequate clinical response, his regimen was changed to rifapentine, levofloxacin, cycloserine, prothionamide, and amoxicillin-clavulanate in February 2013. His sputum became culture negative by August 2014 and has remained so; therefore, he was kept on this regimen for at least 20 months following sputum culture conversion.

DST was performed on isolated strains by the indirect proportion method on LJ slants for rifampin, rifabutin, rifapentine, isoniazid, streptomycin, ethambutol, amikacin, capreomycin, ofloxacin, levofloxacin, prothionamide, and PAS as the initial test and by the critical-concentration method using Bactec MGIT960 (MGIT) growth indicator tubes after reviving the strain from deep-freeze storage for first- and second-line antibiotics according to WHO guidelines and for the following antibiotics (concentrations) that were available for testing: minocycline (1 µg/ml), linezolid (1 µg/ml), and co-trimoxazole (trimethoprim at 38 µg/ml and sulfamethoxazole at 8 µg/ml). The strain derived from patient EF (strain 8897) was resistant to all tested antibiotics except linezolid and co-trimoxazole (Table 1). Furthermore, there was low-level resistance to ethambutol and capreomycin. There were discordant results with respect to susceptibility to PAS and ethionamide: the strain was resistant by the indirect proportion method, but it proved susceptible when tested by MGIT. The strain from patient ZX (strain 11500) was sensitive only to linezolid

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TABLE 1 Phenotype-genotype sensitivities and correlations for XXDR strains^a

Strain (patient details)	Antibiotic(s)	Phenotypic DST result	Gene	Amino acid change(s)
8897 (patient EF; age 64 yrs, male)	Isoniazid	R	<i>katG</i>	R463L, S315T
	Rifampin	R	<i>rpoB</i>	S450L , A1075A
	Rifabutin	R	<i>rpoC</i>	W484G
	Rifapentine	R		
	Ethambutol	R (low level)	<i>embC</i>	R927R
			<i>embB</i>	M306V
			<i>embA</i>	C76C
	Streptomycin	R	<i>rpsL</i>	K43R
			<i>gidB</i>	A205A, E92D
	Pyrazinamide	R	<i>pncA</i>	N
			<i>rpsA</i>	R212R
	Amikacin	R	<i>rrs</i>	N
			<i>eis</i>	N
			<i>tlyA</i>	L11L
	Capreomycin	R (low level)	<i>rrs</i>	N
			<i>eis</i>	N
			<i>tlyA</i>	L11L
	PAS	R ^D	<i>thyA</i>	S125F
	Fluoroquinolones	R	<i>gyrA</i>	S91P , E21Q, S95T, G668D
	Ethionamide	R ^D	<i>ethA</i>	L35P
	Protionamide	R	<i>ethA</i>	L35P
	Minocycline*	R	<i>whiB7^b</i>	N
	Linezolid*	S	<i>rrl</i>	N
	TMP + SMX*	S	<i>dfrA</i>	N
11500 (patient ZX; age 30 yrs, male)	Isoniazid	R	<i>katG</i>	R463L S315T
	Rifampin	R	<i>rpoB</i>	S450L A1075A
	Rifabutin	R	<i>rpoC</i>	N698S
	Rifapentine	R		
	Ethambutol	R	<i>embC</i>	R927R
			<i>embB</i>	M306V
			<i>embA</i>	C76C
	Streptomycin	R	<i>rpsL</i>	K43R
			<i>gidB</i>	A205A, E92D
	Pyrazinamide	R	<i>pncA</i>	Q10P
			<i>rpsA</i>	R212R
	Amikacin	R	<i>rrs</i>	N
			<i>eis</i>	N
			<i>tlyA</i>	L11L
	Capreomycin	R	<i>rrs</i>	N
			<i>eis</i>	N
			<i>tlyA</i>	L11L
	PAS	R	<i>thyA</i>	H75N
	Fluoroquinolones	R	<i>gyrA</i>	A90V , E21Q, S95T, G668D
	Ethionamide	R	<i>ethA</i>	S266R R261W
	Protionamide	R	<i>ethA</i>	S266R R261W
	Minocycline*	R	<i>whiB7^b</i>	N
	Linezolid*	S	<i>rrl</i>	N
	TMP + SMX*	S	<i>dfrA</i>	N

^a Phenotypic DST results from both strains, as measured by MGIT (for all agents except protionamide) and the indirect proportion method (for all agents except those marked by an asterisk [*]) are shown. "Fluoroquinolones" refers to testing of ofloxacin, levofloxacin, and moxifloxacin—all of which gave concordant results in all assays. Agents for which there is phenotype-genotype correlation for "ruled-in" resistance are indicated with boldface characters, as are known resistance-causing mutations. SNPs of unknown significance are indicated with lightface characters. Mutations in *whiB7* and its promoter are also associated with resistance to other antibiotics such as aminoglycosides, lincosamides, and tetracyclines, for which phenotypic DST analysis was not performed in this study. For strain 8897, there were discordant results of phenotypic DST analysis by the proportion and MGIT methods for PAS and ethionamide (both were reported as resistant by the proportion method but were sensitive by MGIT); those results are shown as "R^D." N, no mutations were identified in the gene of interest; TMP + SMX, trimethoprim-sulfamethoxazole.

^b For minocycline, *M. tuberculosis* is inherently resistant to macrolides unless there is a mutation in the *whiB7* gene (2).

and co-trimoxazole and was resistant to the other 17 agents tested (Table 1).

For genomic analysis, the strains were retrieved and genomic DNA was extracted from logarithmically growing cells by stan-

dard methods (1). The DNA was sent for whole-genome sequencing (WGS) on a HiSeq2000 (Illumina) platform with an insertion size of 500 bp and a read length of 90 bp. The mean levels of coverage of the strains were 142× for strain 8897 and 172× for

strain 11500. The raw sequencing reads were processed using a custom perl script to remove adapter contamination. Next, the low-quality bases were trimmed from the reads and the high quality data were used for alignment using bwatools and the H37Rv genome (GenBank accession no. [NC_000962](#)) as a reference. Duplicates were marked and excluded from further analysis, and single nucleotide polymorphisms (SNPs) were called using samtools. We used vcftools software to generate the SNP list in vcf format that was annotated using SnpEff. SNPs that were associated with antibiotic resistance (1) are reported in Table 1 (concordant phenotype-genotype data are shown in bold). The raw sequencing reads of the two strains can be accessed on NCBI's sequence read archive (SRA) database with accession number [SRP058024](#).

Drug resistance is a major threat to attempts to control tuberculosis. Multidrug-resistant tuberculosis (MDR-TB) (tuberculosis resistant to rifampin and isoniazid) was first defined in the reemergence of TB, fueled by the presence of HIV, in New York in the 1980s, with extensively drug-resistant tuberculosis (XDR-TB) (MDR-TB resistant to quinolones and injectable drugs) diagnosed in South Africa in 2006. Of great concern, it is estimated that only a minority (<7%) of cases of multidrug-resistant tuberculosis (MDR-TB) were adequately diagnosed and appropriately treated (2). In the last decade, sporadic cases of tuberculosis resistant to almost all first- and second-line agents have emerged, initially in Italy and Iran (3, 4) and in India (5) and most recently in South Africa (6). In total, these cases of highly resistant tuberculosis number 30, but this is almost certainly an underestimation due to the resource-limiting setting in which the vast majority of tuberculosis cases are endemic.

Here, we report two cases of tuberculosis caused by strains that show phenotypic resistance to an extended panel of antimicrobials, well beyond the definitions of extensively drug-resistant (XDR) tuberculosis, which we have termed extended-spectrum extensively drug-resistant (XXDR) tuberculosis in keeping with prior reports (7–9). Phenotypically, they resemble the cases of “totally drug-resistant tuberculosis” (TDR) reported recently in the literature (3, 4–6). However, since the majority of those strains were not assessed against newer agents such as linezolid (10) and bedaquiline (11) we prefer to use XXDR as the descriptive term rather than TDR, while acknowledging that, for now, XXDR does not have a strict definition.

Although in one of the cases (patient ZX), poor compliance with taking medication was likely a factor in the finding of a successively drug-resistant strain, in the other case, that of patient EF, the first strain isolated and tested for drug resistance was already highly resistant, raising the possibility that the patient was infected by the XXDR strain. There is some debate about whether or not XDR strains exhibit a fitness cost, although MDR strains appear not to exhibit such a cost (12). Regardless, the relative immune paresis of the patient, harboring diabetes and chronic viral hepatitis and possibly an occult hepatoma, probably made the patient more susceptible to infection, even by a strain that might have exhibited reduced fitness. For patient ZX, there were many different results of susceptibility tests performed at other hospitals which we have not independently verified, and we report in Table 1 only the results of the DST performed on the first isolated strain (strain 11500) at the Beijing Chest Hospital, the strain on which

WGS was performed. It is possible that the patient was reinfecting with the XXDR strain, on the background of an initial infection with a more susceptible strain, explaining the widely differing results. The results of WGS analysis of his isolated strain did not, however, suggest coinfection (1).

The use of WGS for diagnosis of drug resistance is not yet feasible in the developing world and has been used only in a research capacity in resource-rich nations (1), although it will likely enter routine practice in such countries within the next few years. There are several advantages to the use of WGS for diagnosis of culture-positive tuberculosis. Extensive information regarding drug susceptibility can be gained within a relatively short time span compared with phenotypic DST, which can take months, where available. Furthermore, phenotypic testing of several agents, including the first-line agent pyrazinamide, is technically difficult, which makes genetic prediction of susceptibility highly attractive. Nonetheless, the genotype-phenotype correlations among many antibiotics are not 100% robust (13). It is easier to “rule in” resistance by identification of a bona fide resistance mutation than to rule it out (1). Moreover, there is considerable uncertainty regarding the significance of several SNPs that have been associated with resistance in clinical samples without extensive *in vitro* experimental verification (e.g., with regard to Rv3728 and capreomycin resistance [14]). Not all SNPs in certain genes highly associated with resistance (e.g., *pncA* and pyrazinamide resistance or *gyrA* and quinolone resistance) are necessarily responsible for drug resistance.

In both strains, WGS failed to identify causative mutations for the resistance to amikacin, kanamycin, and capreomycin. Mutations in *rrs* and *eis* and *tlyA* account for most resistance to these agents, although the proportions of phenotypically resistant strains that lack mutations in these genes have been found to range from 5% to 20% in previous studies (13, 15–17). Furthermore, in strain 8897, no genetic basis for resistance to pyrazinamide was identified, and WGS would have predicted resistance to PAS due to a mutation in *thyA*, whereas phenotypic testing revealed a discordant result. An advantage of WGS is the ability to predict susceptibility to agents for which no DST is locally available, such as cycloserine, clofazimine, bedaquiline, and delamanid (1), to which both strains were found to be susceptible by sequence results. Whether the amoxicillin-clavulanate combination is effective in treatment of tuberculosis is controversial; however, there are reports of its utility (18), and patient ZX has responded well to his regimen, despite only two of the agents (cycloserine and amoxicillin-clavulanate) being potentially effective.

In summary, we report two cases of XXDR within the Beijing region of China. There is evidence of transmissibility for one of the strains, and although WGS may eventually provide a mechanism to rapidly identify drug-resistant tuberculosis to enable more-effective management, further research to validate genotype-phenotype causation is required before it can fully replace phenotypic testing (1, 19).

Nucleotide sequence accession number. Sequences have been deposited in the NCBI SRA database under accession number [SRP058024](#).

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We declare that we have no conflicts of interest. B.J. is a Tsinghua-Janssen Scholar, but Janssen had no role in the design, initiation, or publication of the study.

H.H. and B.J. conceived of the project. H.L., M.U.R.K., Y.G., Y.M., and X.W. performed research. H.L., M.U.R.K., T.Z., H.H., and B.J. analyzed data. H.D. assisted in patient follow-up. H.L., M.U.R.K., and B.J. wrote the paper.

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