

Correspondence

Next-Generation Sequencing for Identifying Pyrazinamide Resistance in *Mycobacterium tuberculosis*

TO THE EDITOR—We read with interest the recent article by Kurbatova et al [1]. Pyrazinamide (PZA) is a key component of multidrug antituberculosis therapy, in both first- and second-line regimens. In their article, Kurbatova and colleagues underscore the public health importance of describing the epidemiology of PZA-resistant *Mycobacterium tuberculosis* (MTB) infection in the United States. Resistance appears to be increasing globally, but its diagnosis is problematic due to standardization issues with PZA drug susceptibility testing [2, 3].

Next-generation sequencing (NGS) of the *pncA* gene of MTB may provide a method to better understand PZA resistance and, consequently, may aid with improving patient care as well. We used NGS to analyze a random sample of 26 clinical isolates from a South African collection of isolates shown to be drug susceptible, multidrug resistant (MDR), or extensively drug resistant by phenotypic testing [4]. Nine of the 26 strains (34.6%) revealed phenotypic PZA resistance. Using Ion Torrent for full-length sequencing of *pncA*, we observed that all 9 contained an amino acid mutation that would confer PZA resistance. Among these was a novel mutation in a stop codon at residue 122.

Furthermore, the growth characteristics of MTB make it difficult to establish PZA resistance using phenotypic drug susceptibility testing procedures [5, 6]. As noted by the authors, “the development of faster, more reliable laboratory methods to detect PZA resistance is a priority.” In another recent use of Ion Torrent full-gene sequencing, we characterized

MDR MTB from 3 immigrants residing in the United States [7]. Genetic characterization of 7 full-length resistance genes was performed within 5 days of the receipt of isolates from the 3 patients (2 MDR and 1 highly resistant strain). One isolate was resistant to isoniazid, rifampin, PZA, and streptomycin, but sensitive to fluoroquinolones both by Ion Torrent sequencing and the Bactec MGIT 960 liquid culture system. NGS revealed a novel Lysine-96-Arg (K96R) *pncA* gene mutation that had not been previously reported.

A specific advantage of NGS is the depth of coverage (ie, deep sequencing) that can be obtained. Phenotypic drug sensitivity testing may not detect resistant MTB subpopulations within a specimen, when these exist. Deep sequencing allows for the detection of heteroresistance in populations of organism that harbor both resistant and susceptible subpopulations. In the case of PZA, for example, an isolate with a low concentration of resistant organisms to the drug may initially be reported as sensitive by phenotypic methods, but during the course of therapy, the PZA-resistant subpopulation may proportionally gain dominance. In a small set of 10 South African isolates, we recently observed conflicting results between phenotypic data (Wayne test and MGIT 960) and traditional Sanger sequencing in 2 isolates. Applying Ion Torrent deep sequencing, we were able to resolve the discrepancy. Two subpopulations of organisms were identified, one with a genotype that confers resistance (a known *pncA* gene mutation) and the other with susceptible wild-type genotype (P.B. Fourie et al, 2013, unpublished data). NGS may assist with resolving discordant results between phenotypic testing and Sanger sequencing, especially in initial

or pretreatment samples where phenotypic testing methods might have a greater probability of missing the diagnosis.

The analysis by Kurbatova et al [1] of the epidemiology of PZA resistance in the United States has put a profound focus on the extent and nature of the problem in this country. Our observations in analyzing MDR tuberculosis specimens in the United States and Africa provide confirmation of the need for continued and more acute surveillance of the problem of PZA resistance, as a clear understanding of the clinical implication of current phenotypic and genotypic resistance test results to this drug is not available. NGS of PZA (and other drug resistance genes) might aid significantly in this regard, and may become a practical tool to guide the treatment of MTB in individuals suspected of carrying drug-resistant MTB from high-prevalence regions around the world.

Note

Potential conflicts of interest. All authors: No reported conflicts.

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