MONDAY-113

Impact of Whole Genome Sequencing of *Mycobacterium tuberculosis* Isolates in a Public Health Laboratory

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Introduction

- *Mycobacterium tuberculosis* (Mtb) remains a pathogen of global importance
- The rate of drug resistant strains (DR-TB) has been increasing since the introduction of antibiotics effective against TB
- Growth-based drug susceptibility testing (DST) can take weeks to months to complete
- Whole genome sequencing (WGS) is capable of performing species identification, spoligotyping, drug resistance profiling, and high resolution genotyping of Mtb strains
- Diagnostic WGS of Mtb was implemented at the Wadsworth Center in February of 2016
- Routine WGS of Mtb has improved drug resistance detection, rapidly providing resistance profiles to eight antibiotics

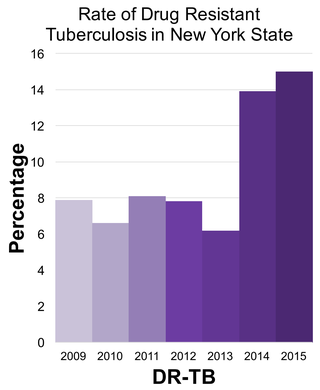
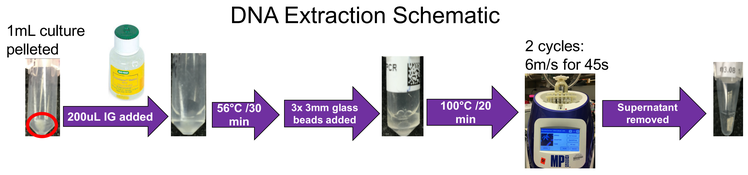
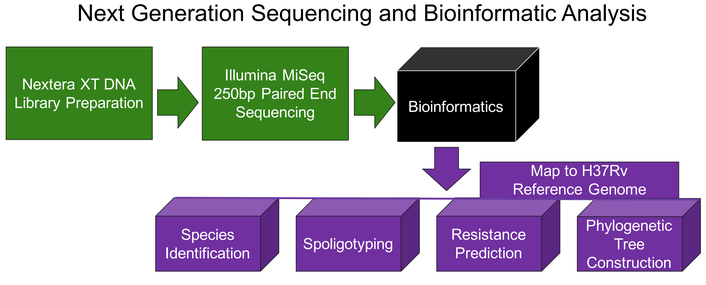


Figure 1. Drug resistant tuberculosis is defined as any strain exhibiting phenotypic resistance to at least one antibiotic.

Methods



- Rapid and cost-effective DNA extraction method (2 hours, $0.52 per sample)
- Yields WGS-suitable DNA from early culture positive isolates
- Nextera XT Library Preparation procedure modified to include 15-cycle PCR indexing step optimized for Mtb
- Bioinformatics pipeline is comprised of multiple modules, each with individualized quality controls



Results

WGS Improves Mtb-Complex Species Identification

Real-time PCR \ WGS	Inconclusive	*M. tuberculosis*	*M. bovis*	*M. bovis*-BCG	*M. africanum*	*M. canettii*
Inconclusive	1	12	1	0	0	0
M. tuberculosis	0	346	0	0	0	0
M. bovis	0	0	6	0	0	0
M. bovis-BCG	0	0	0	11	0	0
M. africanum	1	2	0	0	2	0
M. canettii	0	0	0	0	0	1

Table 1. Multiplex real-time PCR versus Kraken classification of WGS data for 383 strains. Kraken database comprised of all mycobacterial genomes available on NCBI.
Green = Concordant between methods
Blue = Correct by Kraken, incorrect by real-time PCR
Red = Incorrect by Kraken, correct by real-time PCR

Lineages of Mtb Strains Isolated from NYS Patients

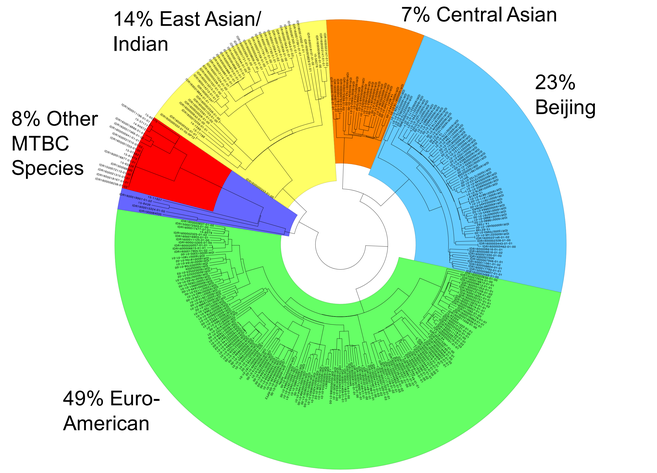


Figure 2. Maximum likelihood phylogenetic tree comprised of strains isolated from new TB patients in New York State from December 2015 through April 2016 (n=324). Non-*M. tuberculosis* strains include *M. bovis* & *M. bovis*-BCG (red) and *M. africanum* & *M. pinnipedii* (purple)

Prevalence of High-Confidence Mutations Used to Predict Antibiotic Resistance

	EMB	FLQ	INH	PZA	RIF	SM	KAN	ETH
Sensitivity	0.79	1.00	0.88	0.90	1.00	0.72	1.00	0.63
Specificity	0.98	1.00	0.99	0.97	0.96	1.00	1.00	0.95
PV-R[a]	0.88	1.00	0.97	0.84	0.84	1.00	1.00	0.89
PV-S[b]	0.97	1.00	0.95	0.98	1.00	0.90	1.00	0.81
Concordance	**0.96**	**1.00**	**0.96**	**0.96**	**0.97**	**0.92**	**1.00**	**0.83**

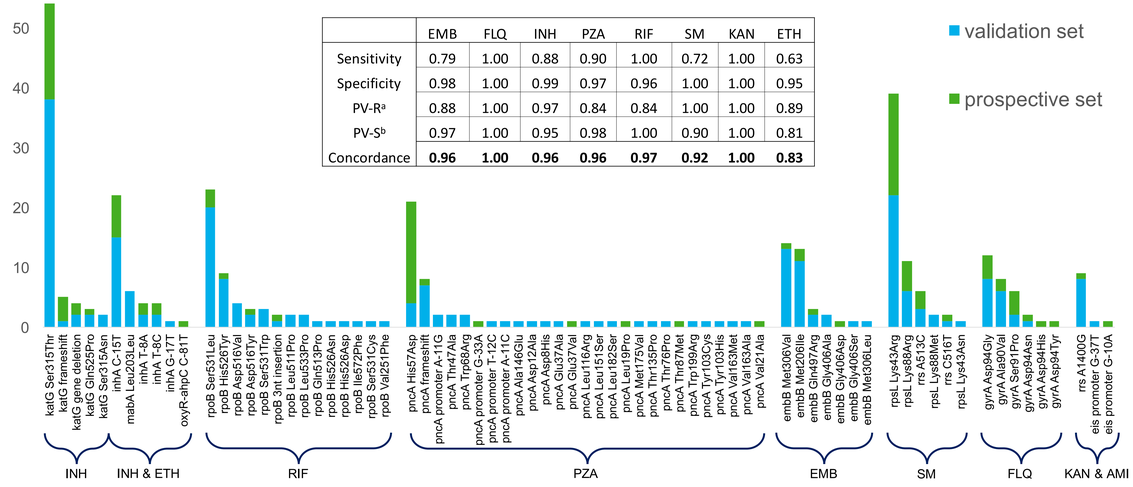


Figure 3. Validation samples were sequenced June 2014 – June 2015 (n=112), prospective samples sequenced July 2015 – May 2016 (n=393). Frameshift mutations and large deletions are only considered high confidence in *rpoB*, *katG*, and *pncA* genes, for RIF, INH, and PZA resistance, respectively. [a] Predictive value for resistance [b] Predictive value for susceptibility
(EMB : Ethambutol, FLQ: Fluoroquinolones, INH: Isoniazid, PZA: Pyrazinamide, RIF: Rifampin, SM: Streptomycin, KAN: Kanamycin, ETA: Ethionamid, AMI: Amikacin)

Turnaround Time

A. WGS Turnaround Time (days to result)		B. Improvement Over Growth-Based DST (days)	
From Extraction	From Culture Positive	1st line drugs[a]	2nd line drugs[b]
7	**14**	**7**	**34**

Table 2. A. Average turnaround time for samples processed February 1 through May 31, 2016. B. Difference in turnaround times between WGS and growth-based DST. DST was performed by Bactec MGIT 960 SIRE and PZA for first line drugs, and agar proportion method for second line drugs
[a] INH, RIF, EMB, PZA, SM [b] FLQ, KAN, AMI, ETH

Conclusions

- Species identification, genotyping, and drug resistance profiling are accomplished within a single WGS assay, streamlining laboratory testing
- Drug resistance profiles are being reported to physicians more rapidly than before
- WGS overcomes limitations of other genotyping methods and is able to definitively identify outbreak clusters and cross-contamination events
- As the genetic bases of resistance are further characterized and understood, this WGS assay will expand to include new targets and mutations to improve drug resistance predictions

Works Cited & Acknowledgements

- Bradley, Phelim et al. "Rapid Antibiotic-Resistance Predictions from Genome Sequence Data for Staphylococcus Aureus and Mycobacterium Tuberculosis." Nature Communications 6 (2015): 10063. PMC. Web.
- Walker, Timothy M et al. "Whole-Genome Sequencing for Prediction of Mycobacterium Tuberculosis Drug Susceptibility and Resistance: A Retrospective Cohort Study." The Lancet. Infectious diseases 15.10 (2015): 1193–1202. PMC. Web.
- Halse, Tanya A., Vincent E. Escuyer, and Kimberlee A. Musser. "Evaluation of a Single-Tube Multiplex Real-Time PCR for Differentiation of Members of the Mycobacterium Tuberculosis Complex in Clinical Specimens ." Journal of Clinical Microbiology 49.7 (2011): 2562–2567. PMC. Web.

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