What Are We Trying to Say Here?

Standardizing Next Generation Sequencing Reports for Tuberculosis

CPTR 2017 Workshop

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JOHNS HOPKINS
CENTER FOR CLINICAL
GLOBAL HEALTH EDUCATION

Jeffrey Tornheim, MD MPH
Clinical Fellow in Infectious Diseases
Johns Hopkins University School of Medicine
tornheim@jhu.edu

Disclosures

- Nothing to disclose

Why does this matter?

- Over the past few years:
  - Increasing recognition of drug resistance
  - Identification of new drugs
  - Expanding access to next generation sequencing
  - Rising numbers of epidemiologists / program managers engaged in studies of strain-relatedness

- UK data suggested cost savings of 7% using NGS rather than present diagnostic workflows
  - NGS has rapidly become a useful clinical tool!

Pankhurst LH, et al. Rapid, Comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study. Lancet Respir Med. 2016

But we don't all speak the same language…

- Epidemiologists
  - Phylogeny, clustering, lineage
  - Contact tracing
- Microbiologists
  - Drug susceptibility
- Geneticists
  - SNPs, indels, nucleotides, amino acids
- Clinicians
  - What can I give my patient?
  - How much can I trust the test?

As a clinician

- An MDR-TB patient has phenotypic DST in process
  - 1st line DST: rifampin resistant by Xpert MTB/RIF
  - Streptomycin, ethambutol susceptible by MGIT
  - 2nd line DST pending

- Genotype shows:
  - embB: Leu355Leu – no effect on ethambutol resistance
  - tlyA: Arg84Gly – effect on capreomycin resistance is unknown

- Can I give him capreomycin?

Bakula Z, et al. Second-line anti-tuberculosis drug resistance and its genetic determinants in multidrug-resistant Mycobacterium tuberculosis clinical isolates. J Microbiol Immunol Infect 2016

What do other users want?

- Online survey of 17 providers (15 in UK)
  - 10 clinicians, 8 epidemiologist or surveillance workers
  - Most felt comfortable interpreting SNPs, SNP-related drug resistance, phylogenetic trees, genomic clusters, and SNP distance

- Most providers wanted speciation, DST, and resistotypes
  - <50% wanted complete epidemiology data

McKee G, et al. COMPASS-TB Report Design Study: First Online Survey. 2016

Structured reports vary widely

- Technical Data
  - Percent mapping to human vs. TB
  - Total # reads, mapped %, coverage %
  - Hetero-resistance, allelic frequency
- Epidemiologic
  - Lineage
  - Phylogenetic trees
  - Identification of outbreak clusters
- Mutation Specific Data
  - Codon change
  - Amino acid change
- Drug Resistance Predictions
  - Drugs of interest
  - Interpretation of mutation:
    - Susceptible vs. Resistant
  - Justification of that interpretation
    - Likelihood of association with resistance
    - MIC range documented with that mutation
    - Confidence in interpretation

So what are we trying to say?

- The goal is to maximize necessary info and exclude everything else
  - Technical data vs. simplicity and readability

- Decisions that need to be made:
  - How to make statements about predicted resistance
  - How to name mutations: nucleotides vs amino acids
  - How much to report new drugs and inconclusive mutations
  - Which pipelines to include
  - How to report hetero-resistance?
  - Should drugs be lumped by class

Who can we model on?

- Stanford HIV database (https://hivdb.stanford.edu/)
- Online tool: enter mutations, spits out interpretation + explanation)

Drug Resistance Interpretation: PR

PI Major Resistance Mutations:	**M46I, L90M**
PI Minor Resistance Mutations:	None
Other Mutations:	I13IV, L63P, H69HY, V77IV

Protease Inhibitors

atazanavir/r (ATV/r)	Intermediate resistance
darunavir/r (DRV/r)	Susceptible
fosamprenavir/r (FPV/r)	Intermediate resistance
indinavir/r (IDV/r)	Intermediate resistance
lopinavir/r (LPV/r)	Low-level resistance
nelfinavir (NFV)	High-level resistance
saquinavir/r (SQV/r)	Intermediate resistance
tipranavir/r (TPV/r)	Susceptible

PR Comments

PIMajor

- M46I/L are nonpolymorphic PI-selected mutations that reduce susceptibility to IDV, NFV, FPV, LPV and ATV when present with other mutations. M46L also reduces susceptibility to TPV.
- L90M is a nonpolymorphic mutation selected primarily by SQV, NFV, IDV and LPV. It reduces susceptibility to each of the PIs except TPV and DRV.

- This is already the current standard of care for HIV

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How Resistance is Predicted	Likelihood Ratios (LRs)	LRs, MIC ranges (where available), Reported Association with Resistance

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$$LR+ = \frac{\Pr(T+|D+)}{\Pr(T+|D-)}$$

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- <u>Threshold for Defining Resistance</u>
  - Resistant = LR ≥ 5
  - No evidence of resistance = LR < 1
  - Possible resistance = LR ≥ 1 and LR < 5

- <u>Confidence Reported by LR Value</u>
  - LR ≥ 10 – High confidence in mutation's association with resistance
  - 5 ≤ LR <10 – More evidence desired to confirm mutation's association with drug resistance
  - 1 ≤ LR < 5 – Inconclusive evidence for mutation's association with drug resistance
  - LR < 1 – No evidence of association between mutation and drug resistance

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- Presentation of both nucleotides and amino acids
  - Coding genes need amino acid:
    - *rpoB, katG, pncA, embB, gyrA, gyrB, rpsL, tlyA, ethA*
  - *Promotor regions need nucleotide changes:*
    - *inhA and eis*

- Certain mutations with the same amino acid change have distinct LRs
  - *rpoB* position 445, His -> Asp by CAC -> GAC (LR 10.00)
  - *rpoB* position 445, His -> Asp by CAC -> AAC (LR 2.67)

- What about insertions and deletions?
  - (T-> TTCGCATGCCGTCACC)

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Inclusion of Newer Drugs	Not included	Include with caveat

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- At present, we are reporting only to following
  - rpoB, katG, inhA, embB, pncA, gyrA, gyrB, rrs, eis
  - Most but not all pncA mutations confer resistance

Whitfield MG, et al. *Mycobacterium tuberculosis pncA* polymorphisms that do not confer pyrazinamide resistance at a breakpoint concentration of 100 micrograms per milliliter in MGIT. J Clin Micro. 2015

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- At present, we are reporting only to following
  - rpoB, katG, inhA, embB, pncA, gyrA, gyrB, rrs, eis
- But whole genome sequencing may tell us more:
  - Ala63Pro mutation in the *atpE* gene associated with 133-fold MIC change for bedaquiline, but not confirmed clinically

Segala E, et al. New mutations in the mycobacterial ATPsynthase: New insights into the binding of the diarylquinolone TMC207 to the ATP Synthase C-Ring Structure. AAC. 2012

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- ReSeqTB pipeline requires:
  - ≥90% of reads map to MTBC by Kraken
  - ≥30X coverage
  - Quality scores ≥Q20
  - Read depth ≥10X

- How do we interpret results from alternative pipelines?
  - At minimum, the pipeline employed must be part of the report

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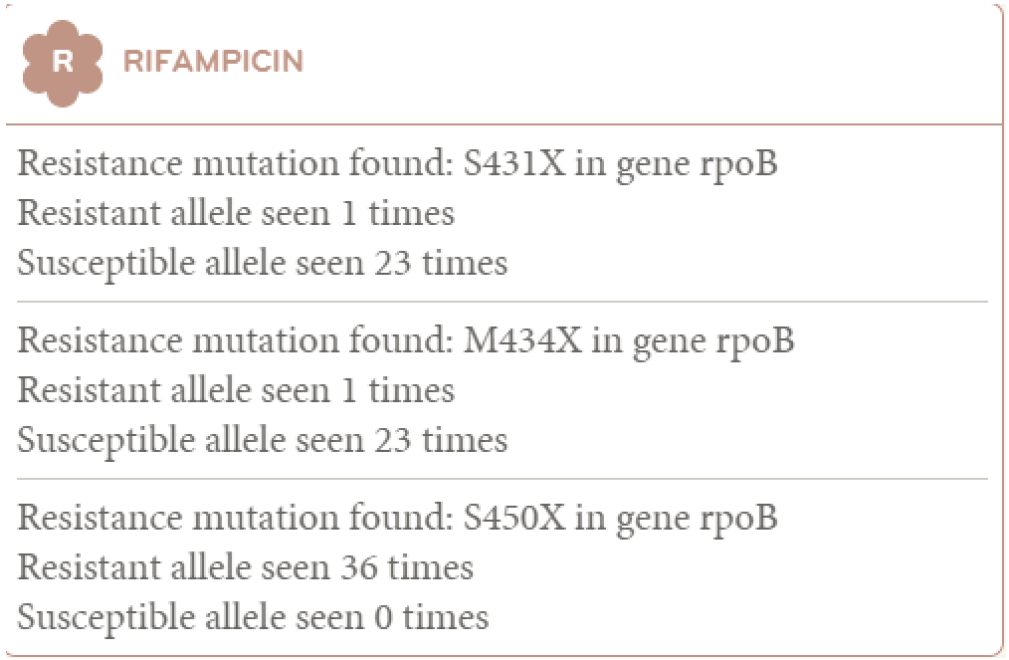
- When mixed populations occur:
  - Reporting frequency may identify minority populations that emerge later
  - May improve confidence in call
  - Unknown significance



- Would you want to know?

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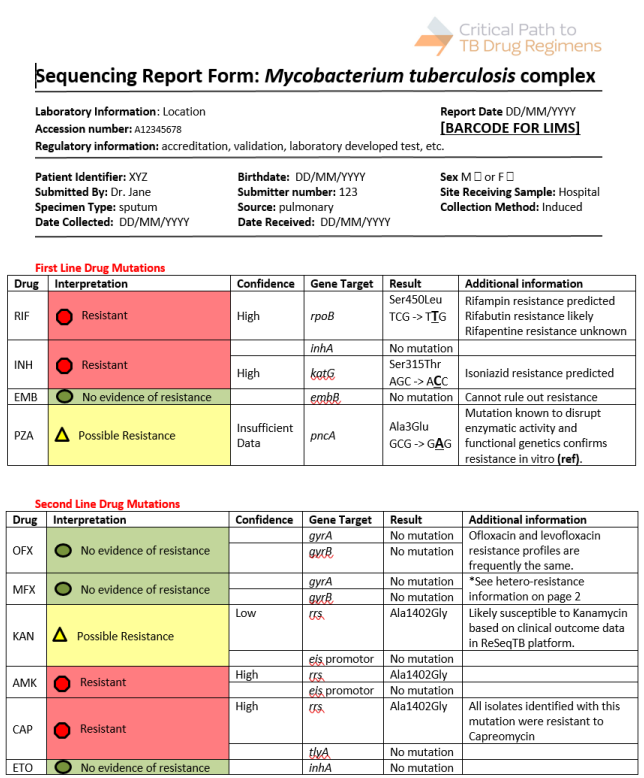
- Do we require distinct information on ofloxacin vs. levofloxacin?
  - What about levofloxacin vs. moxifloxacin?

- What to do if rifampin and rifabutin are discordant?
  - Point mutations (e.g. position 516) may be rifampin resistant but rifabutin susceptible
  - Knowledge of the clinical significance of rifabutin susceptibility in rifampin resistant isolates is insufficient to extrapolate to clinical outcomes

So what are we trying to say?

- The goal is to maximize necessary info and exclude everything else
  - Technical data vs. simplicity and readability
  - Useful to multiple end users

- 6 part report in 2 pages:
  - Basic patient and lab data
  - 1st line DST
  - 2nd line DST
  - Assessment of hetero-resistance
  - Reference to Lineage
  - Explanation of methods



Critical Path to TB Drug Regimens

Sequencing Report Form: *Mycobacterium tuberculosis* complex

Laboratory Information: Location
Accession number: A12345678
Regulatory information: accreditation, validation, laboratory developed test, etc.

Report Date DD/MM/YYYY
[BARCODE FOR LIMS]

Patient Identifier: XYZ
Submitted By: Dr. Jane
Specimen Type: sputum
Date Collected: DD/MM/YYYY

Birthdate: DD/MM/YYYY
Submitter number: 123
Source: pulmonary
Date Received: DD/MM/YYYY

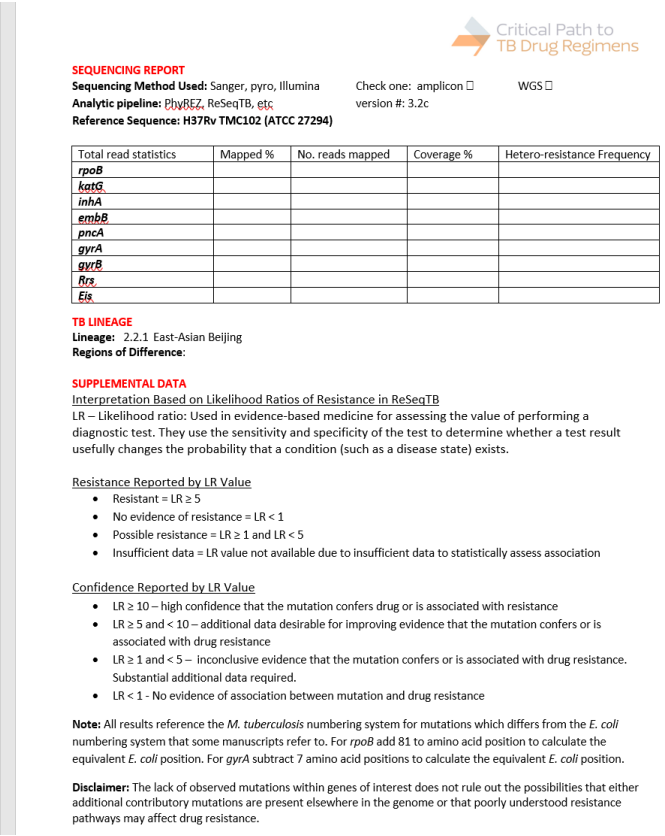
Sex M ☐ or F ☐
Site Receiving Sample: Hospital
Collection Method: Induced

First Line Drug Mutations

Drug	Interpretation	Confidence	Gene Target	Result	Additional information
RIF	Resistant	High	*rpoB*	Ser450Leu TCG -> TTG	Rifampin resistance predicted Rifabutin resistance likely Rifapentine resistance unknown
INH	Resistant		*inhA*	No mutation	
		High	*katG*	Ser315Thr AGC -> ACC	Isoniazid resistance predicted
EMB	No evidence of resistance		*embB*	No mutation	Cannot rule out resistance
PZA	Possible Resistance	Insufficient Data	*pncA*	Ala3Glu GCG -> GAG	Mutation known to disrupt enzymatic activity and functional genetics confirms resistance in vitro **(ref)**.

Second Line Drug Mutations

Drug	Interpretation	Confidence	Gene Target	Result	Additional information
OFX	No evidence of resistance		*gyrA*	No mutation	Ofloxacin and levofloxacin resistance profiles are frequently the same.
			gyrB	No mutation	
MFX	No evidence of resistance		*gyrA*	No mutation	*See hetero-resistance information on page 2
			gyrB	No mutation	
KAN	Possible Resistance	Low	*rrs*	Ala1402Gly	Likely susceptible to Kanamycin based on clinical outcome data in ReSeqTB platform.
			eis promotor	No mutation	
AMK	Resistant	High	*rrs*	Ala1402Gly	
			eis promotor	No mutation	
CAP	Resistant	High	*rrs*	Ala1402Gly	All isolates identified with this mutation were resistant to Capreomycin
			tlyA	No mutation	
ETO	No evidence of resistance		*inhA*	No mutation	



Critical Path to TB Drug Regimens

SEQUENCING REPORT
Sequencing Method Used: Sanger, pyro, Illumina — Check one: amplicon ☐ WGS ☐
Analytic pipeline: PhyREZ, ReSeqTB, etc — version #: 3.2c
Reference Sequence: H37Rv TMC102 (ATCC 27294)

Total read statistics	Mapped %	No. reads mapped	Coverage %	Hetero-resistance Frequency
rpoB				
katG				
inhA				
embB				
pncA				
gyrA				
gyrB				
rrs				
Eis				

TB LINEAGE
Lineage: 2.2.1 East-Asian Beijing
Regions of Difference:

SUPPLEMENTAL DATA
Interpretation Based on Likelihood Ratios of Resistance in ReSeqTB
LR – Likelihood ratio: Used in evidence-based medicine for assessing the value of performing a diagnostic test. They use the sensitivity and specificity of the test to determine whether a test result usefully changes the probability that a condition (such as a disease state) exists.

Resistance Reported by LR Value
- Resistant = LR ≥ 5
- No evidence of resistance = LR < 1
- Possible resistance = LR ≥ 1 and LR < 5
- Insufficient data = LR value not available due to insufficient data to statistically assess association

Confidence Reported by LR Value
- LR ≥ 10 – high confidence that the mutation confers drug or is associated with resistance
- LR ≥ 5 and < 10 – additional data desirable for improving evidence that the mutation confers or is associated with drug resistance
- LR ≥ 1 and < 5 – inconclusive evidence that the mutation confers or is associated with drug resistance. Substantial additional data required.
- LR < 1 - No evidence of association between mutation and drug resistance

Note: All results reference the *M. tuberculosis* numbering system for mutations which differs from the *E. coli* numbering system that some manuscripts refer to. For *rpoB* add 81 to amino acid position to calculate the equivalent *E. coli* position. For *gyrA* subtract 7 amino acid positions to calculate the equivalent *E. coli* position.

Disclaimer: The lack of observed mutations within genes of interest does not rule out the possibilities that either additional contributory mutations are present elsewhere in the genome or that poorly understood resistance pathways may affect drug resistance.

Current approach

- Target-related mutation interpretation for 1st and 2nd line drugs

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- Allows for either probe-based and whole-genome based results

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- Allows for either probe-based or whole genome sequencing

So what are we trying to say?

- Quality control section outlining target mapping & hetero-resistance

Total read statistics	Mapped %	No. reads mapped	Coverage %	Hetero-resistant calls
rpoB				
katG				
inhA				
embB				
pncA				
gyrA				
gyrB				
rrs				
eis				

- Lineage Report
  - **TB LINEAGE**
  - **Lineage:** 2.2.1 East-Asian Beijing
- Explanation of methods section to clarify clinical interpretation
  - LR ≥ 10 – high confidence that the mutation is associated with resistance
  - LR ≥ 5 – 10 – additional data desired to conclude association with resistance
  - LR ≥ 1 – 5 - inconclusive evidence to determine association with resistance
  - LR < 1 – no evidence of association with resistance

Next steps

- Clarify these aspects over the course of this meeting
- Generate an updated reporting template
- Planned WHO meeting to finalize reporting template
- Update as clinical data become available

Thank you for your attention