To MIC or not to MIC, that is the question. Molecular Characterization of Antimicrobial Resistance (AR) for Healthcare in 2019

ORWG Education Session

Dallas, TX

June 15, 2019

Disclosures

- Moderator- April Bobenchik
  - None
- Speaker 1- Trish Simner
  - CheckPoints, bioMérieux, BD Diagnostics, Hardy Diagnostics, Roche Diagnostics, Accelerate Diagnostics, OpGen, CosmosID
- Speaker 2- Paula Snippes
  - None
- Speaker 3- Amy Mathers
  - Accelerate Diagnostics, Antimicrobial Resistance (AMR) Services, VenatoRx, SeLux Diagnostics

Presentation of 3 Different Cases

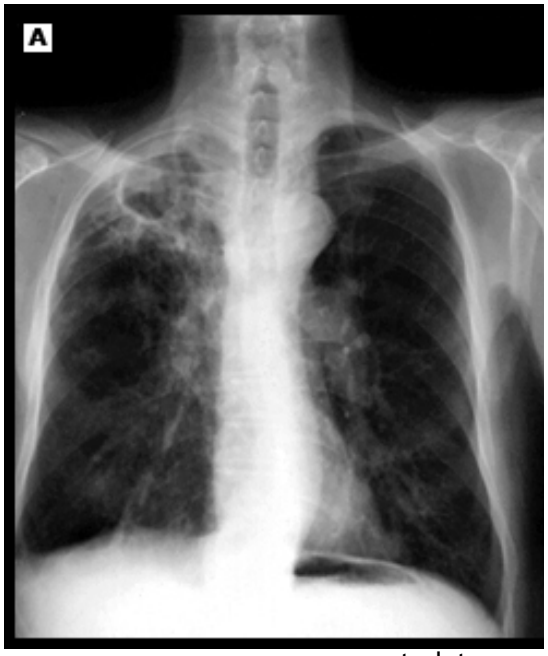
From the perspective of:

- Clinical Microbiology Laboratory
  - How labs are using and reporting molecular AST
  - How to address in antibiograms
- Public Health
  - How PH labs are using molecular testing for outbreak in investigation and surveillance
- Clinician
  - Using results from molecular testing for AR to guide patient management

CASE 1

Case 1

- 80 yr old presenting to ED after generalized weakness leading to a fall, concern for MI
  - Chest X-ray: right apical infiltrate
  - Known positive PPD
- History of present illness:
  - 1 month of cough with sputum production
  - Subjective 40 lbs weight loss
  - Denies shortness of breath/ night sweats
- Social history:
  - Born and raised SE Asia
  - No significant travel within last 12 yrs



www.uptodate.com

Case 1

- Microbiology Cultures
  - Sputum
    - Direct MTB/RIF PCR
    - AFB smear and culture
- Results Timeline
  - Day 0- MTB Detected
    - RIF Detected
  - Day 1- 3+ AFB on smear
  - Day 9- AFB culture positive
    - Pyrosequencing results discordant
  - Day 21- MIC available

Case 1

Molecular AR

Clinical Lab

Performed at Public Health Lab

Xpert MTB/RIF	
RIF R	Detected

INH	Suggests S to INH
katG	No mutation
inhA	No mutation
ahpC	No mutation

RIF	Probably S to RIF
rpoB (426-440)	No mutation
rpoB (441-452)	No mutation
rpoB (170)	No mutation

MIC Testing

Antimicrobial	Result
Isoniazid – 0.1 µg/ml	Susceptible
Rifampin – 1 µg/ml	Susceptible
Ethambutol – 5 µg/ml	Susceptible
Pyrazinamide – 100 µg/ml	Susceptible
Moxifloxacin – 0.25 µg/ml	Susceptible
Amikacin – 1.5 µg/ml	Susceptible
Capreomycin – 3 µg/ml	Susceptible
Ethionamide – 5 µg/ml	Susceptible
Rifabutin – 0.5 µg/ml	Susceptible
Kanamycin – 3.5 µg/ml	Susceptible

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From a Lab Director's Perspective

3 Different Scenarios Encountered:

1. Genotype correlates with phenotype – Woohoo!

2. Detection of a AMR resistance marker with a susceptible AST profile

3. Lack of detection AMR resistance marker and a resistant AST profile

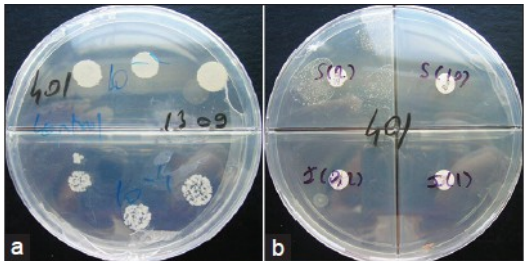
The Complexities of Molecular Methods for AMR

- Genotype to phenotype correlations can be complex and depends on the methods, targets, regions of targets, databases utilized for the different organisms/antimicrobial agents being evaluated

- Lab directors should educate themselves in the methods and limitations to be prepared to answer questions and suggest further testing (if applicable) to the clinical team

cAST vs mAST: The Advantage of Time!

- Culture dependent Antimicrobial Susceptibility Testing (cAST)
  - Agar proportion, broth systems (MGIT or VersaTREK) Sensititre microtitre dilution method
  - TAT: average of 2-3 weeks… up to 4+ weeks



Karabulut et al, Indian J Med Microbiol, 2014.

- Molecular AST(mAST)
  - Directly from raw specimens or sediment
  - More rapid results!
    - Earlier effective treatment, improved patient outcomes and reduction in transmission

- Complementary Methods
  - Not all relevant mutations are known
  - cDST of rifampin (RIF) imperfect & mDST may yield more information

MCM, 12th Ed, 2018.

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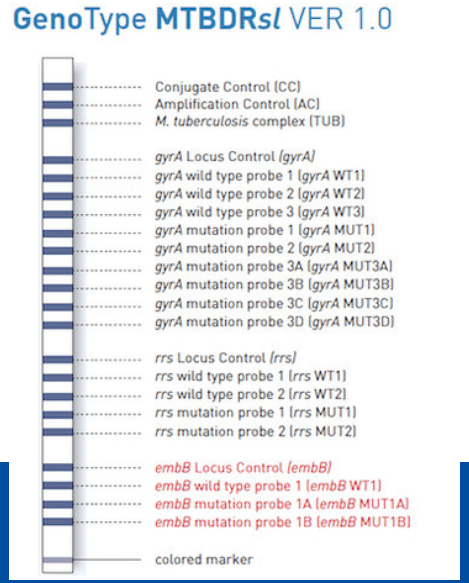
Probe-based mAST

- Cepheid Xpert MTB/RIF assay
  - *rpoB* gene targeted to predict rifampin susceptibility
  - Can detect the presence or absence of mutations
    - Includes silent mutations leading to false-resistance and disputed mutations leading to discordant results (1-19% of *rpoB* mutations)
  - CDC recommends confirmation by sequencing when mutations are detected
    - Especially in a low prevalence setting
- Line Probe Assays (LPAs)
  - Identifies a few commonly seen mutations while identified unidentified mutations by missing the wild-type bands



MCM, 12th Ed, 2018.; CLSI M24, 3rd Edition

JCM
Journals.ASM.org

DNA Sequencing for Confirmation of Rifampin Resistance Detected by Cepheid Xpert MTB/RIF Assay

Allison J. McAlister, Jeffrey Driscoll, Beverly Metchock

Division of Tuberculosis Elimination, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Atlanta, Georgia, USA

- February 2011 to July 2014
- Isolates referred to CDC to confirm RIF resistance
  - 64 isolates evaluated – Xpert, cDST & *rpoB* sequencing
  - 39/40 with known mutations tested RIF resistant
  - 9 with disputed mutations – 3 R and 6 S by cDST
  - 12 with silent mutations (19%)

TABLE 1 Frequency of *rpoB* mutations identified in study samples

RRDR result	No. (%) of samples with DST result: RIF^r	RIF^s	Total
Ser531Leu	26	0	26 (41)
His526Tyr	3	0	3 (5)
His526Asp	2	1	3 (5)
Ser531Trp	2	0	2 (3)
Gln513Leu	1	0	1 (2)
Asp516Val	1	0	1 (2)
His526Arg	1	0	1 (2)
Phe514PhePhe	1	0	1 (2)
His526Arg/Cys/Tyr[a]	1	0	1 (2)
Leu511Pro[b]	0	2	2 (3)
Asp516Tyr[b]	0	2	2 (3)
His526Ser[b]	0	1	1 (2)
Leu533Pro[b]	0	1	1 (2)
His526Leu[b]	1	0	1 (2)
Leu511Pro and Asp516Ala[b]	1	0	1 (2)
Ser512Arg and His526Asn[b]	1	0	1 (2)
Asp516Glu and Ser522Leu	1	0	1 (2)
Asp516Gly and Ser522Leu	0	1	1 (2)
Phe514Phe[c]	0	11	11 (17)
Leu521Leu[c]	0	1	1 (2)
No mutation	0	2	2 (2)
Total	42 (66)	22 (34)	64
Mutations associated with RIF^r	39	1	40 (63)
Mutations associated with low-level RIF^r	3	6	9 (14)
Silent mutations	0	12	12 (19)

[a] Mixed peaks were observed (CAC > YRC).
[b] Mutation associated with low-level RIF^r (i.e., disputed mutation).
[c] Silent mutation.

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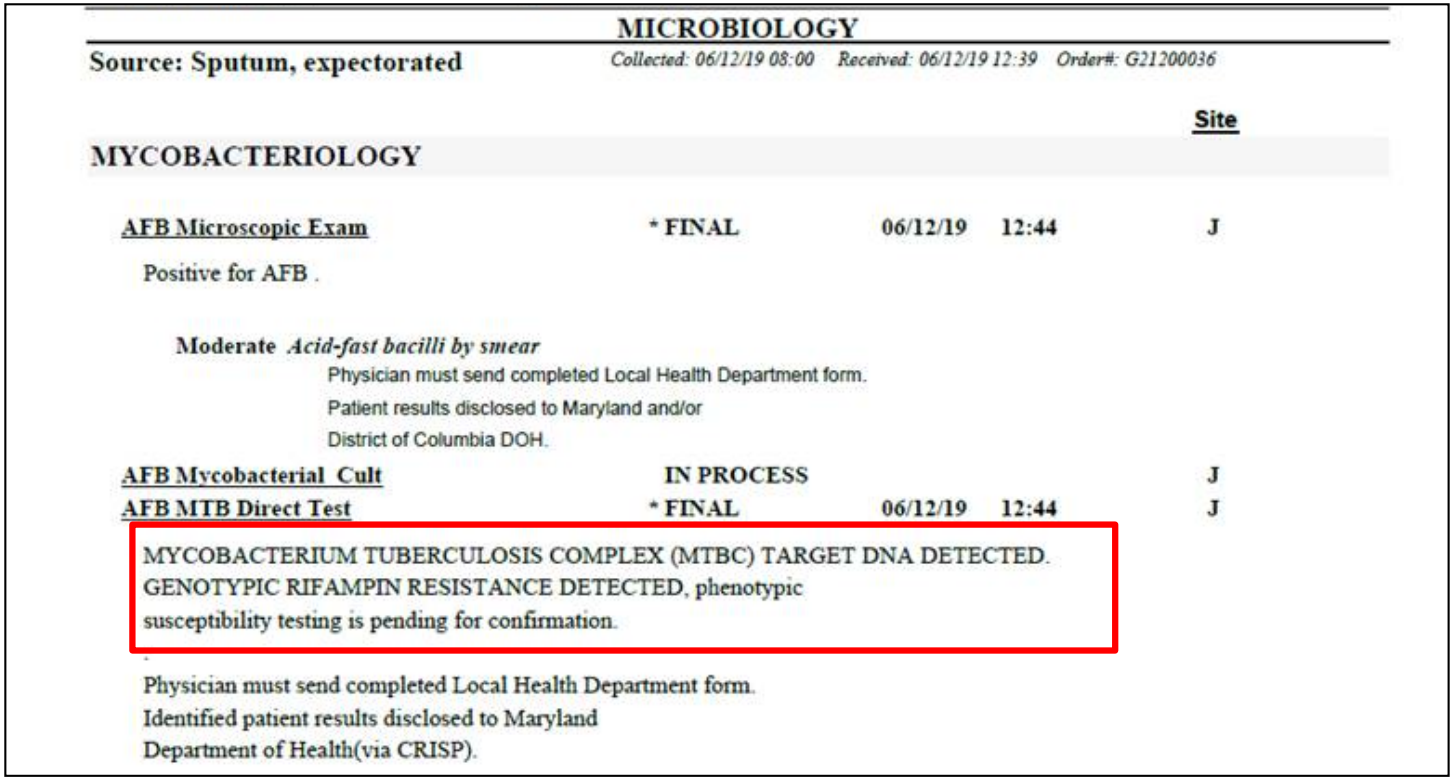
Reporting



MICROBIOLOGY

Source: Sputum, expectorated *Collected: 06/12/19 08:00 Received: 06/12/19 12:39 Order#: G21200036*

				Site
MYCOBACTERIOLOGY				
AFB Microscopic Exam	*** FINAL**	**06/12/19**	**12:44**	**J**

Positive for AFB .

Moderate ***Acid-fast bacilli by smear***

Physician must send completed Local Health Department form.
Patient results disclosed to Maryland and/or
District of Columbia DOH.

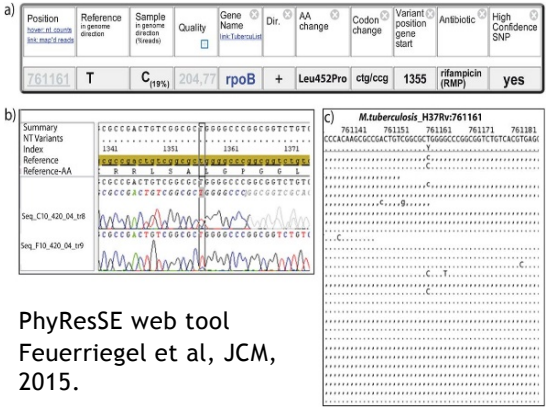
AFB Mycobacterial Cult	**IN PROCESS**			**J**
AFB MTB Direct Test	*** FINAL**	**06/12/19**	**12:44**	**J**

MYCOBACTERIUM TUBERCULOSIS COMPLEX (MTBC) TARGET DNA DETECTED.
GENOTYPIC RIFAMPIN RESISTANCE DETECTED, phenotypic
susceptibility testing is pending for confirmation.

Physician must send completed Local Health Department form.
Identified patient results disclosed to Maryland
Department of Health(via CRISP).

Sequencing-Based mAST

- Becoming more commonplace
- Sanger, pyrosequencing, targeted next-generation sequencing (tNGS) or whole genome sequencing (WGS)
  - Available through State Health Labs & the CDC
- Allows users to recognize silent mutations, disputed mutations, mutations that confer different levels of resistance
  - Association of MICs with AMR mutations are evolving
  - Incomplete picture → not all resistance mechanisms are known or the interaction between mechanisms
  - Highly curated databases are important!
    - In-house validated, UVP, Mykrobe, TBDReaMDB, MUBII-TB-DB



PhyResSE web tool
Feuerriegel et al, JCM, 2015.

Accuracy of Predictions Compared to cDST

Antimicrobial Agent	MDDR – PCR & Sanger			PCR & Pyrosequencing		
	Targets	Sens	Spec	Targets	Sens	Spec
Rifampin	*rpoB*	97.1%	97.4%	*rpoB*	96.3%	100%
Isoniazid	*inhA* + *katG*	86.0%	99.1%	*inhA* + *katG* + *ahpC-oxyR*	87.6%	100%
Ethambutol	*embB*	78.8%	94.3%	*embB*	-	-
Pyrazinamide	*pncA*	86.0%	95.9%	*pncA*	-	-
Fluoroquinolones	*gyrA*	79.0%	99.6%	*gyrA*	87%	100%
Kanamycin	*rrs* + *eis*	86.7%	99.6%	*rrs*	85.7%	100%
Amikacin	*rrs*	90.9%	98.4%	*rrs*	100%	99%
Capreomycin	*rrs* + *tlyA*	55.2%	91.0%	*rrs*	100%	99%

https://www.cdc.gov/tb/topic/laboratory/mddrusersguide.pdf; https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/MDL-Pyrosequencing-for-XDR-TB-Screening.pdf

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How Do You Resolve Discrepant Results?

Table 4. CLSI M24, 3rd Edition.

Issue	Problem	Suggested Action
Poor reproducibility	PZA: False-resistance using broth dilution systems RIF: False-resistance by probe-based molecular methods	PZA: repeat phenotypic AST and sequence *pncA* gene RIF: Perform cAST or sequencing
Silent mutations	Detection of SNV that due not cause changes in amino acid or functional change in the gene product	Confirm with cDST or sequencing methods that enable specific DNA sequence to be determined
Disputed mutations	A mutation that does not confer drug resistance by cAST at the critical concentration. Mutations may result in slightly elevated MICs than the control or low-level resistance.	Consider determining MIC or using sequencing methods that identify specific DNA sequence. Consider a combined phenotypic and genotypic methods if low-level resistance is suspected. Simultaneous mutations can lead to resistance.
Heteroresistance	Sanger >10 % resistant bacteria to detect a mixed pop or LPA slightly more sensitive (>5% pop to detect)	Consider a combined approach of phenotypic and genotypic testing.

Reporting Considerations

- Absence of a mutation does not rule-out resistance
  - Suggests susceptibility or Likely susceptible or Cannot rule out resistance
  - Add accuracy estimates
- Steps should be taken to resolve “false-resistant” results
  - Sequencing methods or cDST
  - Determine is truly false-resistant due to silent mutation or a disputed mutation resulting in low-level resistance which may be associated with poorer outcomes

CDC Molecular Detection of Drug Resistance (MDDR) Report

Conventional Drug Susceptibility Test in progress.

Locus (region) examined*	Result	Interpretation (based on in-house evaluation of 550 clinical isolates)
rpoB (RRDR)	**Mutation:** TCG>TTG; Ser531Leu	Rifampin resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are RMP-R.)
inhA (promoter)	No mutation	Isoniazid resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are INH-R.)
katG (Ser315 codon)	**Mutation:** AGC>ACC; Ser315Thr	
embB (Met306,Gly406)	**Mutation:** TTC>TCC; Phe330Ser Silent mutation: GCG>GCA; Ala376Ala	Effect of the Phe330Ser mutation on Ethambutol resistance is unknown. Cannot rule out ethambutol resistance. (79% of EMB-R isolates in our in-house evaluation of 550 clinical isolates have a mutation other than the one detected at this locus.) The Ala376Ala mutation is a synonymous (silent) single-nucleotide polymorphism (SNP) and does not result in an amino acid change and is not considered clinically significant.
pncA (promoter, coding region)	**Mutation:** ACG>ATG; Thr87Met Silent mutations: GCC>GCT; Ala28Ala TTC>TTT; Phe94Phe	Effect of this mutation on pyrazinamide resistance is unknown. This mutation has been reported to be associated with PZA resistance in the literature. The Ala28Ala and Phe94Phe mutations are synonymous (silent) single-nucleotide polymorphisms (SNPs) and do not result in an amino acid change and are not considered clinically significant.
gyrA (QRDR)	No mutation	Cannot rule out fluoroquinolone resistance. (80% of FQ-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
rrs (1400 region)	No mutation	The effect of the tlyA mutation on capreomycin is unknown. Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 550 clinical isolates: • 91% of AMK-R isolates have a mutation in the rrs locus; • 87% of KAN-R isolates have a mutation in either the rrs locus or the eis locus; • 55% of CAP-R isolates have a mutation in either the rrs locus or the tlyA locus.)
eis (promoter)	No mutation	
tlyA (entire ORF)	**Mutation:** GAA>AAA; Glu59Lys	

Known resistance mutation

Disputed & Silent mutation

No known mutation

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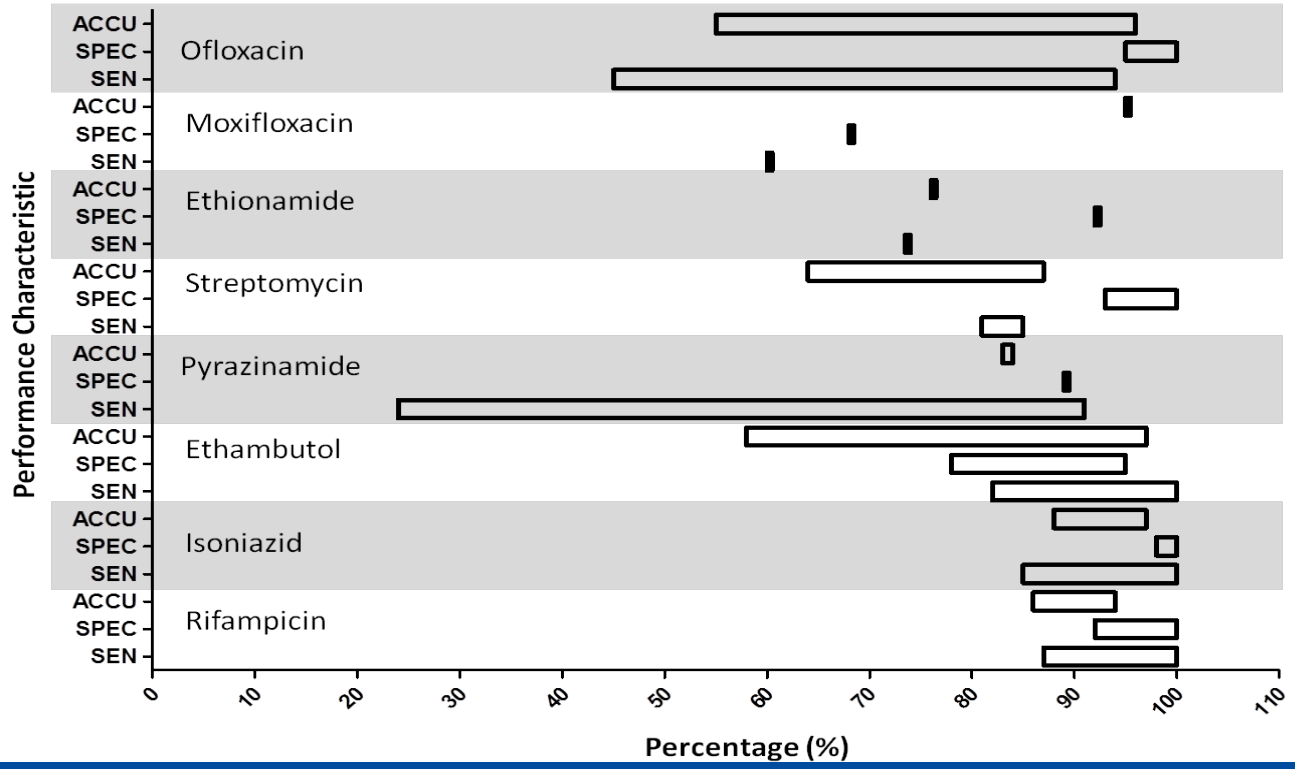
What's Next? WGS To Predict AST

Comparison of Sensitivity and Specificity from Rule-Based Approaches and Accuracy from Model-Based Approaches



Yee & Simner, Advances in Mol Path, *In press*.

What to do clinically when rif resistance comes back on your patient?

Like many things in medicine…

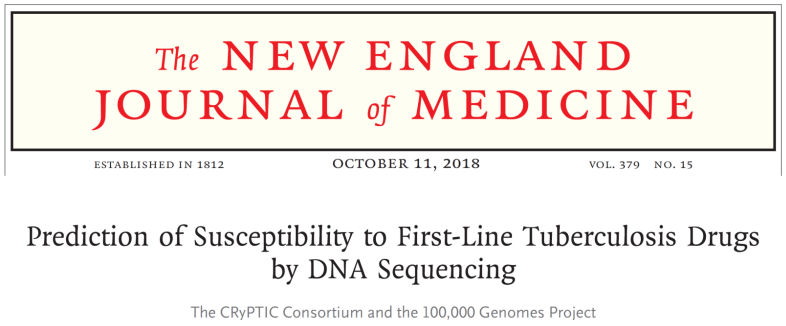
It depends on the patient, but it is a challenging result if right or wrong

When listed as Rifampin resistant…

- Need to do molecular triage with an experienced TB clinician
- Assess the likelihood of resistance in the patient
- Need to get sequencing/AST done as soon as possible
- Knowing the rest of the susceptibility profile is helpful
- Hold off on initiation of treatment if possible until can be resolved in low risk patients

Subset of the recent WGS MTb project

n=10,290 original but enriched for MDR Tb
14 countries
All phenotypic to WGS comparison
38 total discrepancies



- 4397 isolates from low risk resistance areas*
- Among these isolates, 335 (7.8%) were isoniazid-resistant compared to 3294 (33%) for the rest of the data set

*German, Italian, Dutch, and U.K. collections

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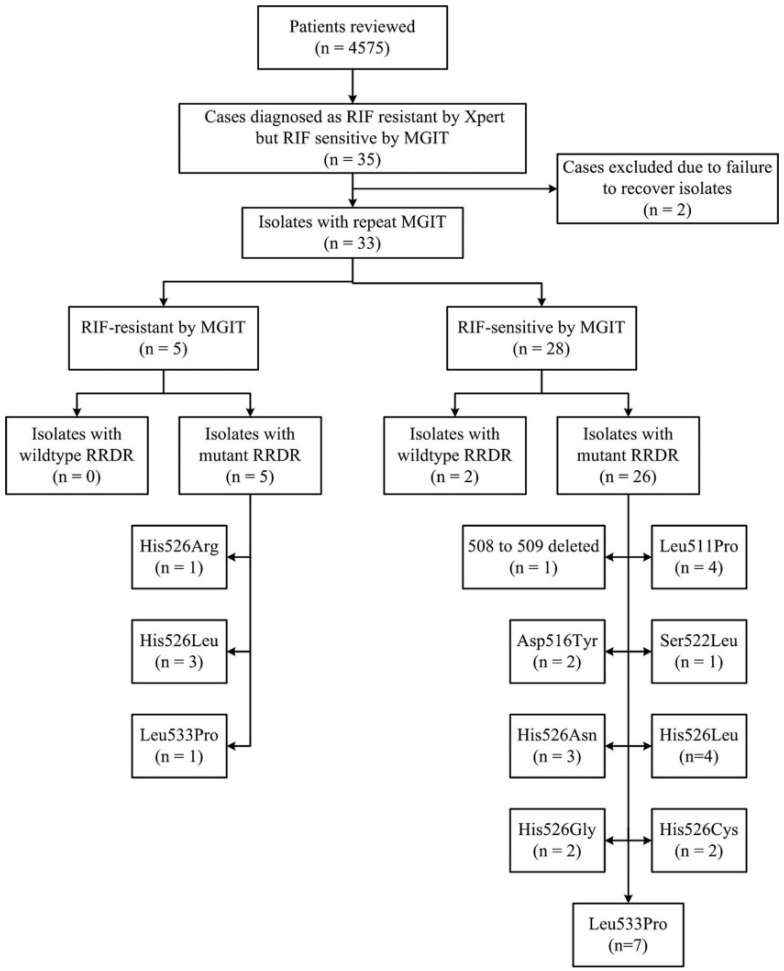
WGS resistance prediction specificity depends on prevalence

Difference in performance compared to whole data set ($p \leq 0.05$)

Table 2. Prediction of Phenotypes of Resistance or Susceptibility to Individual Drugs.*

Analysis and Drug	Resistant Phenotype					Susceptible Phenotype					Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Sensitivity, All†	Specificity, All†	NGP	RP
	R	S	U	F	Total	R	S	U	F	Total								
	number of isolates										*percent*							
WGS, all isolates																		
Isoniazid	3067	90	93	44	3294	65	6313	215	117	6710	97.1 (96.5–97.7)	99.0 (98.7–99.2)	97.9 (97.4–98.4)	98.6 (98.3–98.9)	93.1	94.1	4.7	32.9
Rifampin	2743	69	7	84	2903	85	6763	232	147	7227	97.5 (96.9–98.1)	98.8 (98.5–99.0)	97.0 (96.3–97.6)	99.0 (98.7–99.2)	94.5	93.6	4.6	28.7
Ethambutol	1410	81	94	55	1640	468	6835	781	70	8154	94.6 (93.3–95.7)	93.6 (93.0–94.1)	75.1 (73.0–77.0)	98.8 (98.5–99.1)	86.0	83.8	10.2	16.7
Pyrazinamide	863	82	117	77	1139	204	6146	197	108	6655	91.3 (89.3–93.0)	96.8 (96.3–97.2)	80.9 (78.4–83.2)	98.7 (98.4–99.0)	75.8	92.4	6.4	14.6
WGS, unenriched‖																		
Isoniazid	314	8	9	4	335	15	3770	104	90	3979	97.5 (95.2–98.9)	99.6 (99.3–99.8)§	95.4 (92.6–97.4)¶	99.8 (99.6–99.9)§	93.7	94.7	4.8	7.8
Rifampin	126	0	0	9	135	31	3958	103	116	4208	100.0 (97.1–100.0)	99.2 (98.9–99.5)**	80.3 (73.2–86.2)§	100.0 (99.9–100.0)§	93.3	94.1	5.2	3.1
Ethambutol	72	1	0	0	73	47	3711	458	36	4252	98.6 (92.6–100.0)	98.7 (98.3–99.1)§	60.5 (51.1–69.3)§	100.0 (99.8–100.0)§	98.6	87.3	11.4	1.7
Pyrazinamide	109	6	4	6	125	30	4003	14	58	4105	94.8 (89.0–98.1)	99.3 (98.9–99.5)§	78.4 (70.6–84.9)	99.9 (99.7–99.9)§	87.2	97.5	1.9	3.0

RpoB mutation positive yet rifampin susceptible isolates from a high risk group?



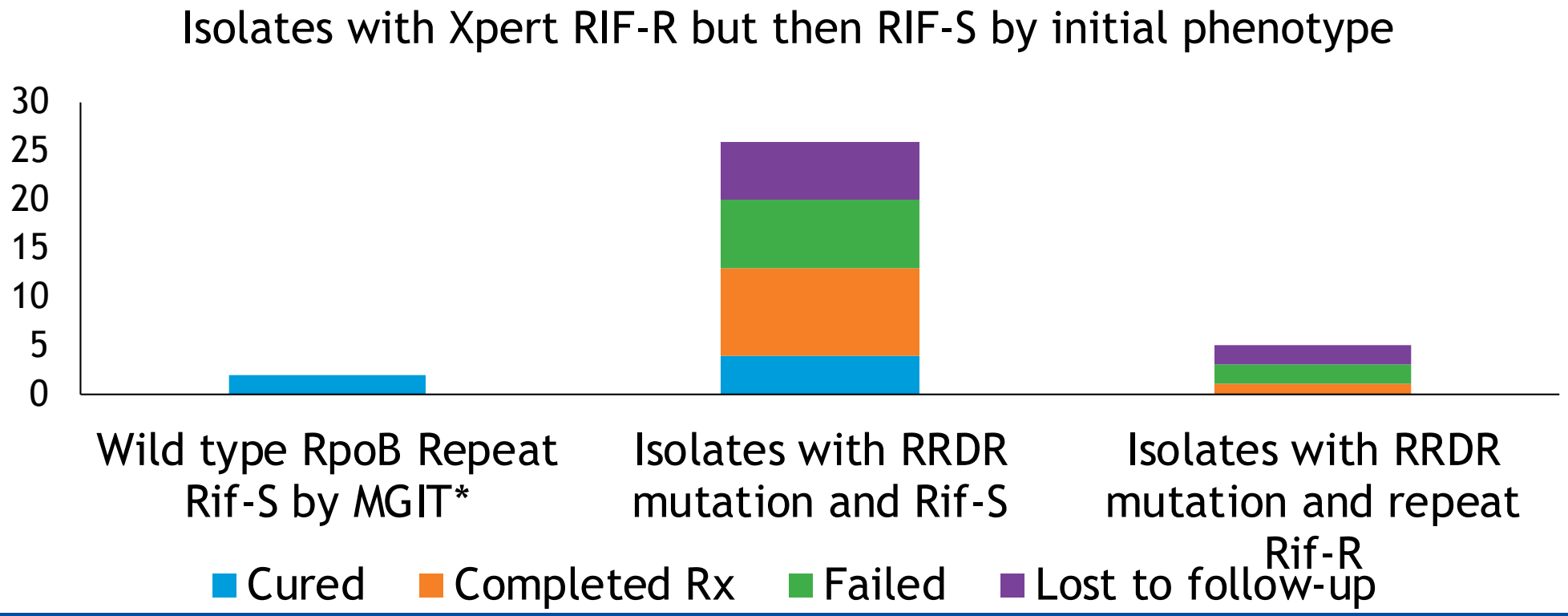
China-Rif-R on Xpert but Rif susceptible MIC

n=33 patients

2-false positive genotype
5-Rif resistant on repeat phenotypic
26-Rif susceptible on repeat phenotypic but most with elevated MICs
27/31 with INH resistance

Peilei Hu et al. J. Clin. Microbiol. 2019;
doi:10.1128/JCM.01707-18

The genotype may be better at predicting failure?



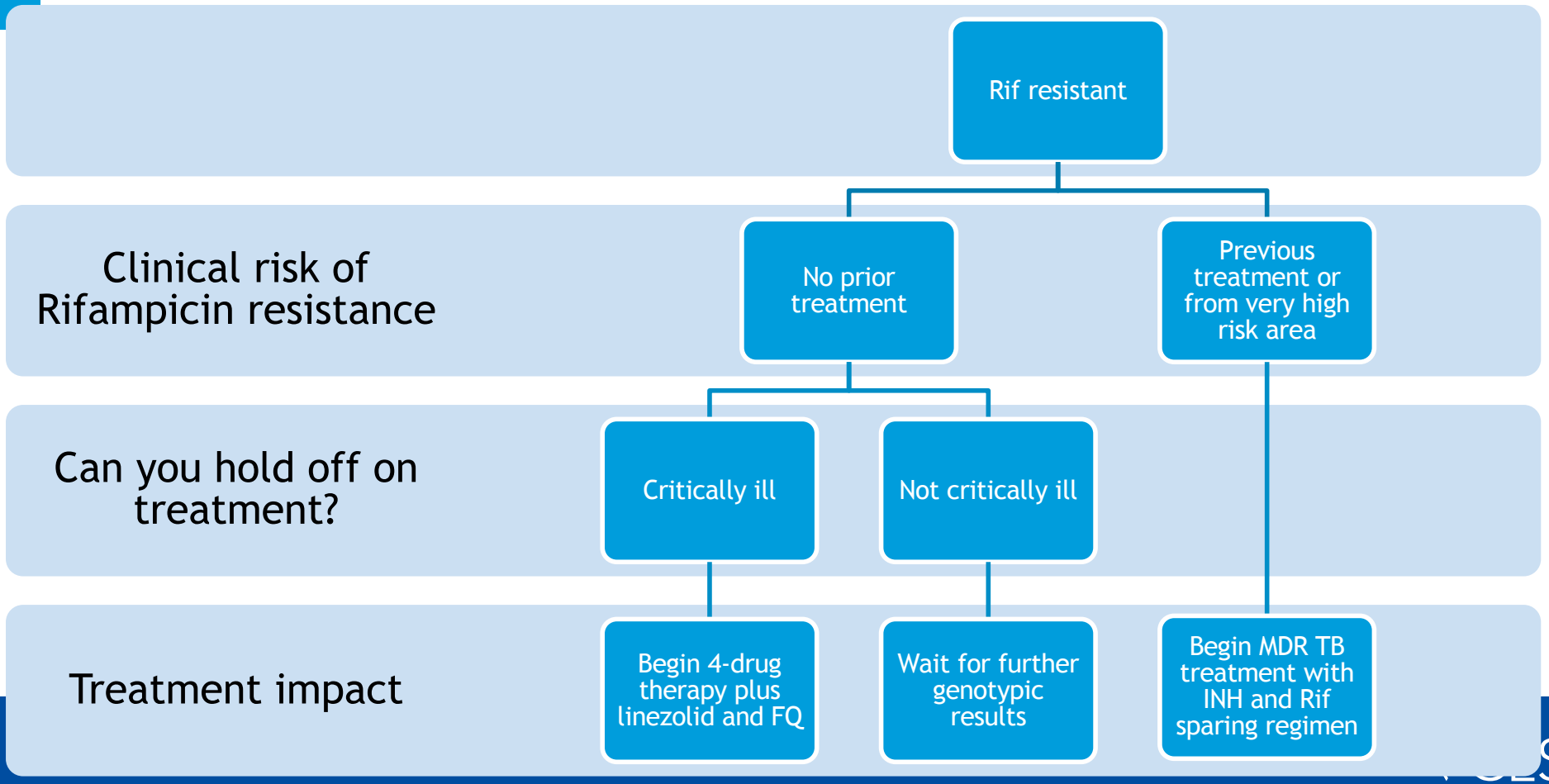
*Both had very high C_T

How different is the regimen really?

- If really rifampin resistant then likely MDR (i.e. INH resistant)
- Completely different regimen with higher failure rates, longer duration and greater toxicity
- Can’t use rifampin and bedaquiline together

Groups & steps	Medicine
Group A: Include all three medicines	levofloxacin *OR* moxifloxacin
	bedaquiline[2,3]
	linezolid[4]
Group B: Add one or both medicines	clofazimine
	cycloserine *OR* terizidone
Group C: Add to complete the regimen and when medicines from Groups A and B cannot be used	ethambutol
	delamanid[3,5]
	pyrazinamide[6]
	imipenem–cilastatin *OR* meropenem[7]
	amikacin (*OR* streptomycin)[8]
	ethionamide *OR* prothionamide[9]
	p-aminosalicylic acid[9]

Decision tree and molecular triage



Case 1: MDDR – Molecular Detection of Drug Resistance (CDC)

BACKGROUND

- DNA sequencing for detection of mutations most frequently associated with rifampin and isoniazid resistance
- Additional testing – based on algorithm to ID mutations associated with resistance to the most effective 2nd line drugs
  - Fluorquinolones, amikacin, kanamycin, and capreomycin

https://www.cdc.gov/tb/topic/laboratory/default.htm

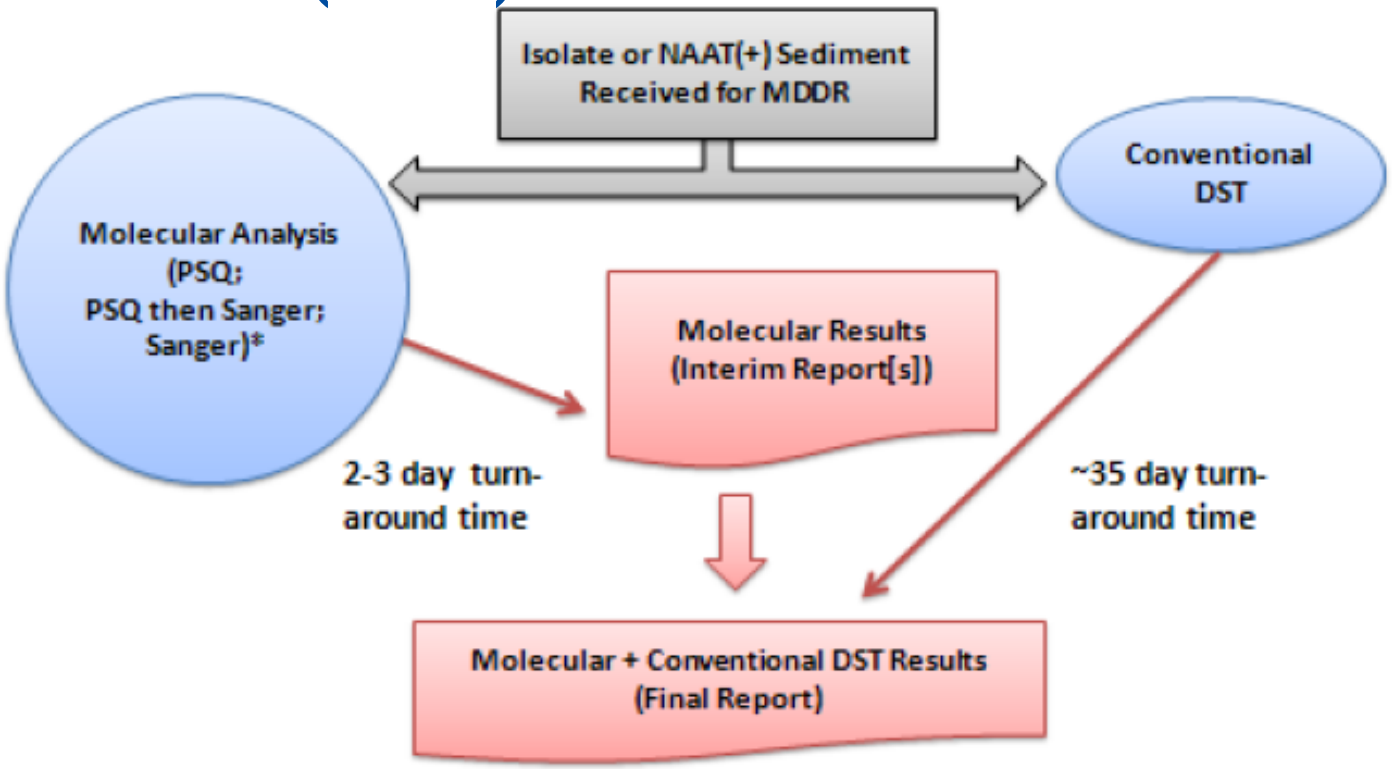
Case 1: MDDR – Molecular Detection of Drug Resistance (CDC)

CRITERIA FOR SUBMISSION

- Isolate or NAAT + sediment (not raw specimen)
- Patients at high risk (Rifampin-R, MDR TB)
  - From pop. with high rates of drug resistance
  - Exposed to drug resistance case
  - Failing therapy
- Rifampin resistance
  - Conventional or molecular test performed by submitter

https://www.aphl.org/conferences/Documents/2017%20TB%20Conference%20Presentations/09Metchock.pdf

Case 1: MDDR – Molecular Detection of Drug Resistance (CDC)



https://www.aphl.org/conferences/Documents/2017%20TB%20Conference%20Presentations/09Metchock.pdf

Case 1 – MDDR Result from CDC

Locus (region) examined*	Result	Interpretation (based on in-house evaluation of 550 clinical isolates)
rpoB (RRDR)	**Silent Mutation:** C>T; Phe514Phe	**The mutation detected is a synonymous (silent) single-nucleotide polymorphism (SNP) which does not result in an amino acid change and is not considered clinically significant. Probably Rifampin susceptible.**
inhA (promoter)	No mutation	**Cannot rule out INH resistance. (86% of INH-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at one or both of these loci.)**
katG (Ser315 codon)	**No mutation**	
embB (Met306,Gly406)	**No mutation**	**Cannot rule out ethambutol resistance. (79% of EMB-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this loci.)**
pncA (promoter, coding region)	**No mutation**	**Cannot rule out PZA resistance. (86% of PZA-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this loci.)**
gyrA (QRDR)	No mutation	Cannot rule out fluoroquinolone resistance. (80% of FQ-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
rrs (1400 region)	No mutation	Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 550 clinical isolates: • 91% of AMK-R isolates have a mutation in the rrs locus; • 87% of KAN-R isolates have a mutation in either the rrs locus or the eis locus; • 55% of CAP-R isolates have a mutation in either the rrs locus or the tlyA locus.)
eis (promoter)	No mutation	
tlyA (entire ORF)	No mutation	

Case 1: Public Health and TB Whole Genome Sequencing

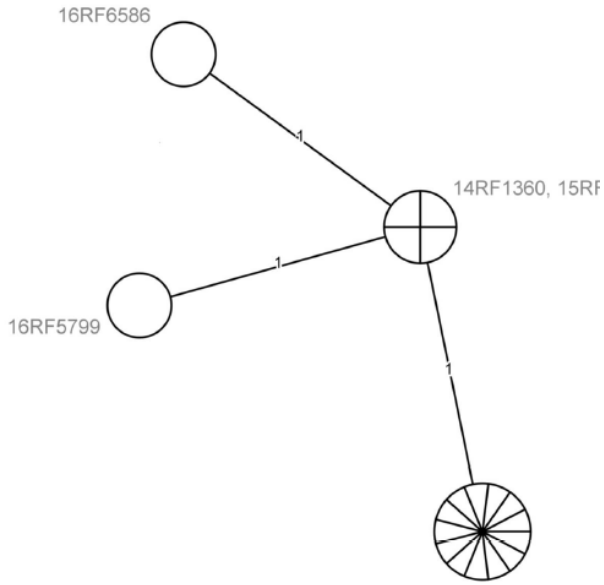
Whole-genome single nucleotide polymorphism (wgSNP) analysis

- Further assess the genetic relatedness of the isolates.
- Isolates closely related indicate possible recent transmission
- wgSNP analysis expands coverage of the genome to ~90% (i.e., compared to ~1% coverage with conventional genotyping).
- Resulting phylogenetic tree can be used to target and inform epidemiologic investigation of these cases.

MDH TB Epidemiology; email correspondence CDC

Case 1: Public Health and TB Whole Genome Sequencing

- TB bacteria grow slowly; generally don't mutate at a high rate
- Each TB patient infected with a diverse population of TB bacteria; WGS map represents the most common TB bacterial profile



MDH TB Epidemiology; email correspondence CDC

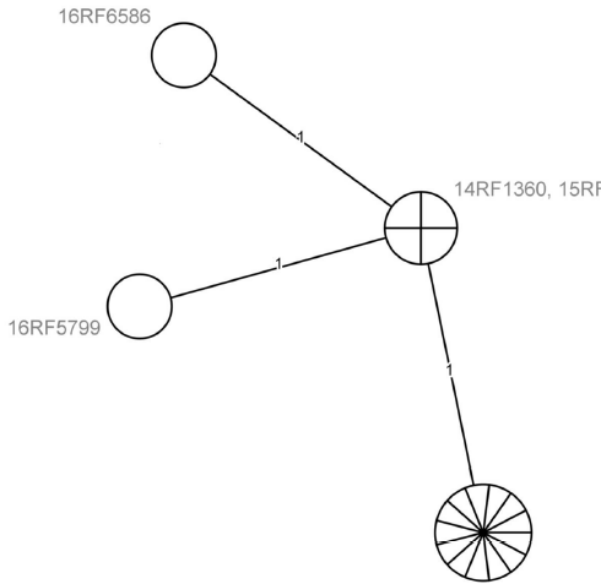
Case 1: Public Health and TB Whole Genome Sequencing

General guidelines

- Circles represent M.tb isolates from different patients
- DNA base pair (bp) differences represented by length of line between isolates
- Connections between isolates don't imply transmission
- Patients with identical strains represented in same circle (node)
- Currently no definitive guideline for "closely related" strain - generally < 4 bp difference (4-5 bp = "gray area")

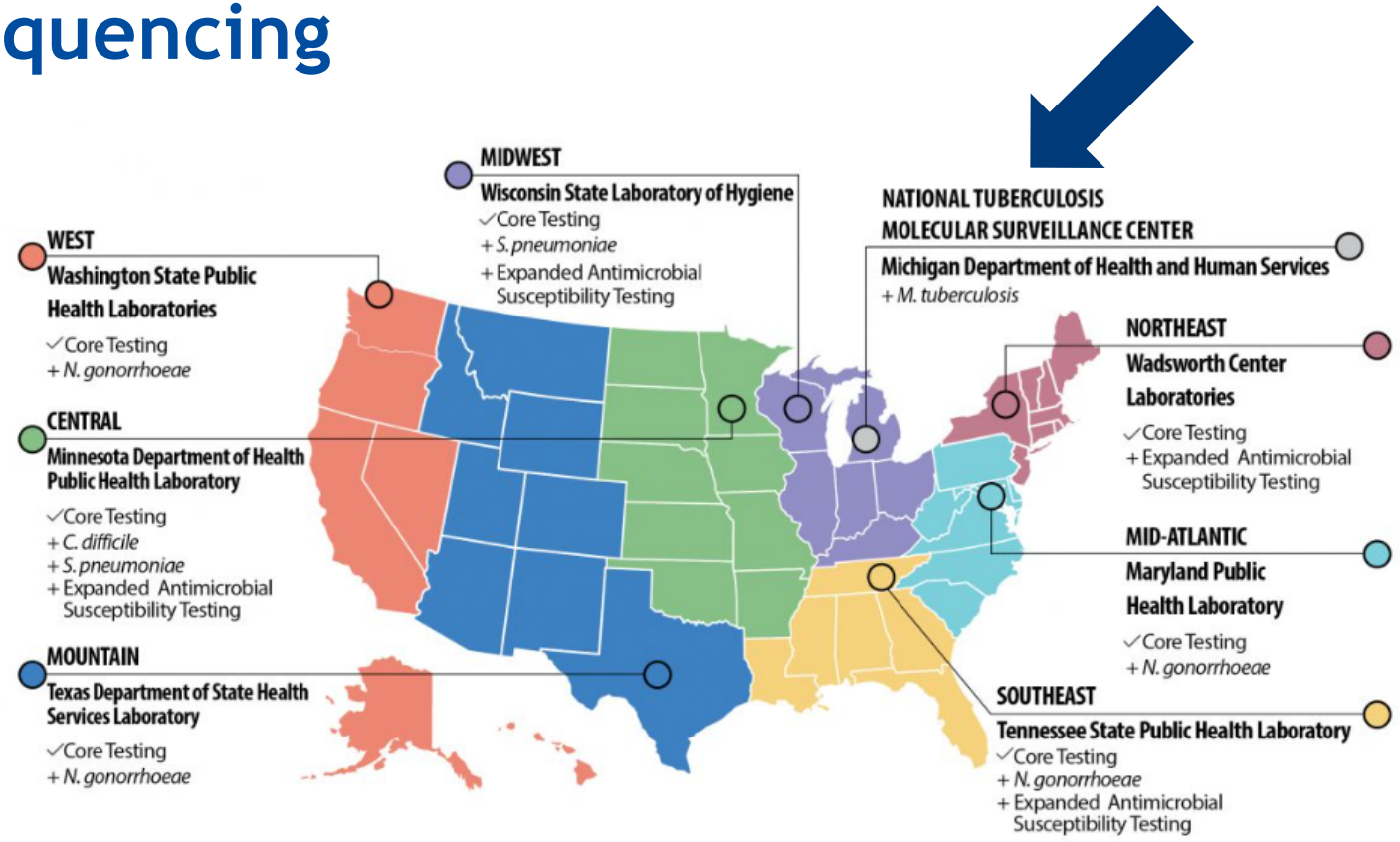
WGS results need to be considered together with clinical and epi data

- Epi-linked patients with closely related strains from WGS analysis are considered to be in same chain of transmission



MDH TB Epidemiology; email correspondence CDC

Case 1: Public Health and TB Whole Genome Sequencing



https://www.cdc.gov/drugresistance/laboratories/AR-lab-network-testing-details.html

Case 1: Public Health and TB Whole Genome Sequencing

AR LABORATORY NETWORK REGIONAL LAB – Michigan

- National Tuberculosis (TB) Molecular Surveillance Center Michigan Department of Health and Human Services
- Beginning in March 2018, implemented universal whole genome sequencing (WGS) of all culture-confirmed cases of tuberculosis (TB) in the United States
  - WGS maps automatically generated for certain larger clusters; others upon request
- The WGS data helps detect and support outbreak investigations, and will allow for nationwide molecular surveillance of drug-resistant TB.

https://wwwn.cdc.gov/ARInvestments/PDFDocs/Michigan-CDC-AR-Investments.pdf

Case 1: Public Health and TB Whole Genome Sequencing

AR LABORATORY NETWORK REGIONAL LAB – Michigan

- Has sequenced more than 6,000 isolates of *Mycobacterium tuberculosis*.
- Eventually will completely replace conventional M.tb genotyping methods based on only ~1% of genome (spoligotyping and MIRU analysis)

https://wwwn.cdc.gov/ARInvestments/PDFDocs/Michigan-CDC-AR-Investments.pdf