Discordant Growth-Molecular Rifampin Resistance Diagnostic Challenges and Treatment Outcomes

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NAR
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Case 1 (The Uncle)

- 20s year old male from Mexico diagnosed with smear-positive, pulmonary TB
- PSQ results
  - *katG* mutation: "Associated with INH resistance"
  - *rpoB* mutation 526AAC: "Not associated with RIF of RFB resistance."
- Growth-based susceptibility testing: INH resistance only
- Treated with RIF, EMB, PZA for 9 months
- End of treatment sputum: smear and culture-positive

FAILED

Case 2 (Niece)

- 8 year old with TB osteomyelitis and pulmonary TB
- Growth-based susceptibility testing : INH resistance, Rifampin sensitive
- Treated for 12 months with RIF, EMB, PZA
- 4 months after completion: recurrent ankle pain with positive AFB fluid aspirate from joint
- PSQ showed *rpoB* 526AAC mutation

RELAPSED

Case 3 (Nephew)

- 1 year old male with meningeal and pulmonary TB
- Growth-based susceptibility testing: INH resistance
- Completed 12 months of treatment with RIF, EMB, PZA, FQN
- Poor neurologic outcome

Why did these patients fail or relapse?

What do these mutations mean that are RIF sensitive on growth based susceptibility testing?

BACKGROUND

Susceptibility Testing for Rifampin

- **Rifampin is the cornerstone to TB treatment**
  - Treatment for rifampin resistance requires a longer duration of therapy
  - Therefore accurate rifampin drug susceptibility testing is crucial
- **Diagnosing drug resistant TB is difficult**

Drug Susceptibility Testing

- **Growth-based susceptibility tests = culture-based susceptibility tests = phenotypic susceptibility tests = DSTs**
- **Molecular-based tests for drug resistance**
  - "Molecular susceptibility tests"
  - "Genotypic susceptibility tests"

Growth-based susceptibility testing

- **Reference standard for drug-susceptibility testing**
- **Differences in test method**
- **Differences in drug concentration**
- **Long turn around time**

Antimicrobial Agent	Typical MIC (μg/ml) for susceptible strains	Concentration in serum (μg/ml)	Medium and concentration (μg/ml)				
			7H10 low/high	BACTEC 460TB 12B low/high	MGIT 960 low/high	VersaTREK low/high	MB/BacT ALERT 3D
Primary Agents							
• INH	0.05-0.2	7	0.2/1	0.1/0.4	0.1/0.4	0.1/0.4	1
• RIF	0.5	10	1	2	1	1.0	1
• PZA	20	45	NR*	100	100	300	200
• EMB	1-5	2-5	5/10	2.5/7.5	5	5.0/8.0	2
Secondary Agents							

Benefits of Molecular Tests for Drug Resistance

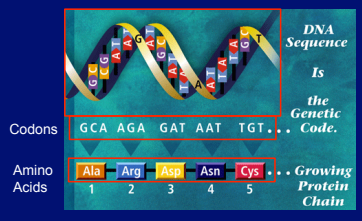
- **Reduced time to detection of resistance**
  - Time from empiric (first-line) treatment to MDR treatment 40 days less (median)
  - Less transmission
  - Less acquired resistance while DSTs pending
  - Less ineffective LTBI treatment given to contacts

Banerjee et al, J Clin Micro, 2010

Molecular Tests for Drug Resistance

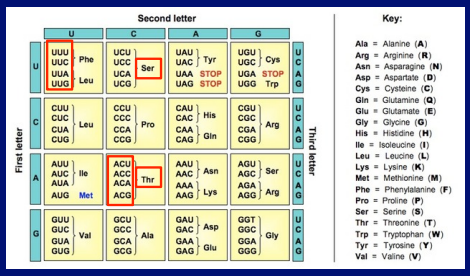
- **Non-sequencing (reports presence of a mutation)**
  - Molecular beacons: Cepheid Xpert MTB/Rif -- FDA authorized
  - Line probes: Hain MTBDRplus and MTBDRsl, Innogenetics INNO-LiPA Rif.TB
- **Sequencing (reports specific mutation)**
  - Pyrosequencing (PSQ)
  - CDC's Molecular Detection of Drug Resistance (MDDR)
  - Whole genome sequencing
- **98% RIF resistance within 81 base pair "hot spot" of a gene called *rpoB***

How Does Molecular Testing Work?



- **Mutations in single bases can lead to resistance**

Genetic Code



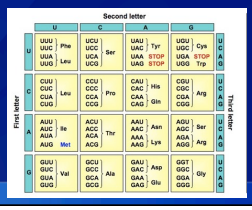
Types of mutations

Silent

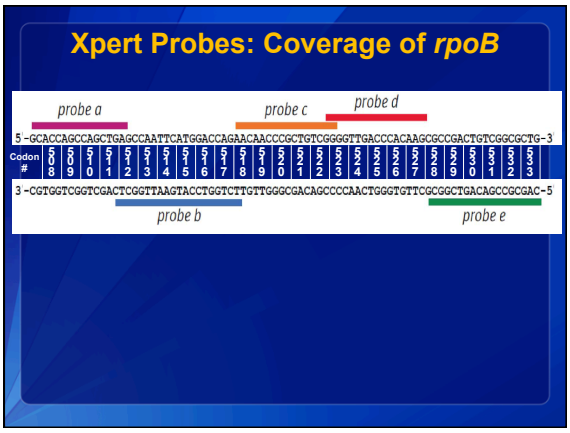
- Codon change
- No amino acid change
- Not associated with drug resistance generally

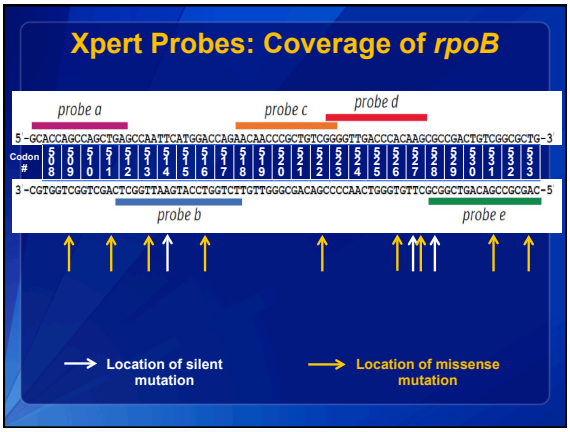
Missense

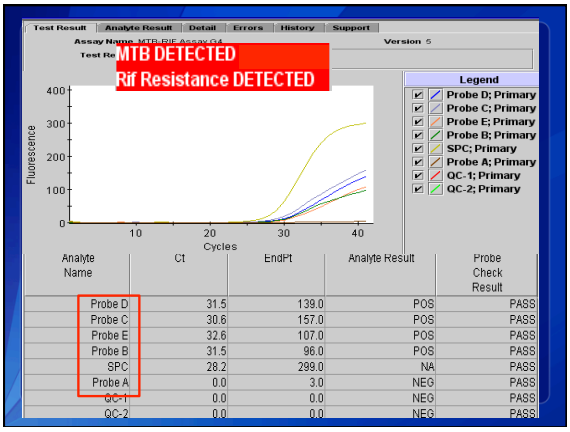
- Codon change
- Amino acid change
- Some are associated with resistance



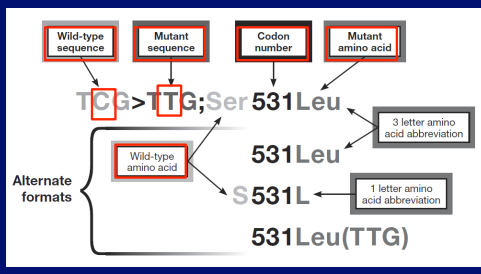
NON-SEQUENCING BASED MOLECULAR TESTING


Xpert Probes: Coverage of rpoB
probe a
probe c
probe d
5 -GCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG- 3
Codon #
508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533
3 -CGTGGTCGGTCGACTCGGTTAAGTACCTGGTCTTGTTGGGCGACAGCCCCAACTGGGTGTTCGCGGCTGACAGCCGCGAC- 5
probe b
probe e


Xpert Probes: Coverage of rpoB
probe a
probe c
probe d
5 -GCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG- 3
Codon #
508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533
3 -CGTGGTCGGTCGACTCGGTTAAGTACCTGGTCTTGTTGGGCGACAGCCCCAACTGGGTGTTCGCGGCTGACAGCCGCGAC- 5
probe b
probe e
Location of silent mutation
Location of missense mutation


Test Result
Analyte Result
Detail
Errors
History
Support
Version 5
MTB DETECTED
Rif Resistance DETECTED
Legend
Probe D; Primary
Probe C; Primary
Probe E; Primary
Probe B; Primary
SPC; Primary
Probe A; Primary
QC-1; Primary
QC-2; Primary
Fluorescence
Cycles
Analyte Name
Ct
EndPt
Analyte Result
Probe Check Result
Probe D 31.5 139.0 POS PASS
Probe C 30.6 157.0 POS PASS
Probe E 32.6 107.0 POS PASS
Probe B 31.5 96.0 POS PASS
SPC 28.2 299.0 NA PASS
Probe A 0.0 3.0 NEG PASS
QC-1 0.0 0.0 NEG PASS
QC-2 0.0 0.0 NEG PASS

Understanding sequence-based results



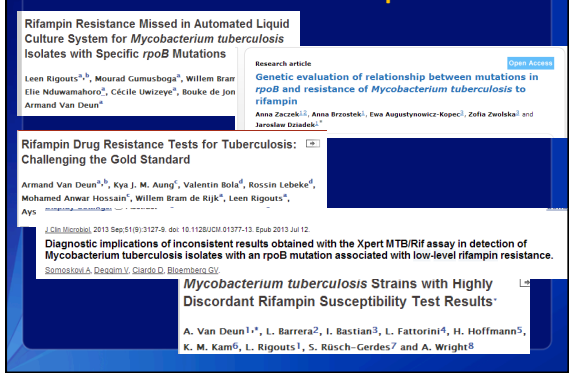
Using example of a mutation in *rpoB*

Discordant growth-molecular susceptibility testing

Definition

- Growth-based susceptibility testing often is RIF sensitive
- Molecular testing shows a missense mutation
- What is the meaning of mutations that are still sensitive on growth based testing?

Studies of Discordant Rifampin Results



Rifampin Resistance Missed in Automated Liquid Culture System for *Mycobacterium tuberculosis* Isolates with Specific *rpoB* Mutations

Leen Rigouts[a,b], Mourad Gumusboga[a], Willem Bram Elie Nduwamahoro[d], Cécile Uwizeye[a], Bouke de Jon Armand Van Deun[a]

Research article — Open Access

Genetic evaluation of relationship between mutations in *rpoB* and resistance of *Mycobacterium tuberculosis* to rifampin

Anna Zaczek[1,2], Anna Brzostek[1], Ewa Augustynowicz-Kopec[3], Zofia Zwolska[3] and Jarosław Dziadek[1,*]

Rifampin Drug Resistance Tests for Tuberculosis: Challenging the Gold Standard

Armand Van Deun[a,b], Kya J. M. Aung[c], Valentin Bola[d], Rossin Lebeke[d], Mohamed Anwar Hossain[c], Willem Bram de Rijk[a], Leen Rigouts[a], Ays

J Clin Microbiol. 2013 Sep;51(9):3127-9. doi: 10.1128/JCM.01377-13. Epub 2013 Jul 12.

Diagnostic implications of inconsistent results obtained with the Xpert MTB/Rif assay in detection of Mycobacterium tuberculosis isolates with an rpoB mutation associated with low-level rifampin resistance.

Somoskovi A, Deggim V, Ciardo D, Bloemberg GV.

Mycobacterium tuberculosis Strains with Highly Discordant Rifampin Susceptibility Test Results

A. Van Deun[1,*], L. Barrera[2], I. Bastian[3], L. Fattorini[4], H. Hoffmann[5], K. M. Kam[6], L. Rigouts[1], S. Rüsch-Gerdes[7] and A. Wright[8]

Summary of literature on discordant *rpoB* mutations

- **Discordant mutations associated with high rate of treatment failures**
- **Many were also INH resistant**
- **Conclusions**
  - Perhaps growth-based DST should not be reference standard
  - Discordant mutations have clinical significance
- **Studies from high incidence settings; mostly from cases being retreated**

California Molecular Detection Laboratory Experiences

Methods

- **Laboratory criteria:**
  - Growth-based susceptibility testing showed RIF susceptible AND
  - *rpoB* missense mutation determined by pyrosequencing
- **Eligibility criteria: all case-patients during 2003–2013**
- **Demographic data were abstracted from the California TB registry**
- **Clinical information including medical history, treatment and outcomes were abstracted from medical records**
- **TBGIMS used to confirm patients were not infected with new strain**

Methods
Definitions

- **Relapse: recurrent episode of TB after having been previously treated for TB with a documented cure or treatment completion.**
- **Failure: positive sputum culture after having a documented sputum culture conversion during the same course of TB treatment or failure to convert sputum cultures after 5 months of therapy**

California Discordant Cases
Results

- **3330 specimens tested**
  - 413 specimens that had an *rpoB* mutation (12.4%)
  - 16 of 413 specimens (3.9% or 0.5% of the all specimens tested) had an *rpoB* mutation associated with discordant growth-molecular susceptibility testing
- **Seven discordant mutations identified: 511Pro (CCG, n = 3), 516Phe (TTC, n = 1), 526Asn (AAC, n = 6), 526Ser [AGC (n = 2), TCC (n = 1)], 526Cys (TGC, n = 1), and 533Pro (CCG, n = 2).**

Results

- **14 of 16 (88%) were resistant to isoniazid (INH)**
  - 6 also resistant to ethambutol (EMB) and/or pyrazinamide (PZA)
  - 2 resistant to fluoroquinolone
- **5 patients (31%) relapsed or failed**
  - 1 with 511Pro
  - 4 with 526Asn

CA Discordant Cases

rpoB Mutation	Growth based DST	Treatment Regimen	Treatment Outcome
511Pro (CCG)	H	2 HRZE **9 RZE**	**Relapsed**
	None	6 HRZE	Cured
	H, ETO, SM, EMB	6 EZ, MFX, AMK, LNZ, RFB 18 EZ, MFX, LNZ, RFB	Cured

CA Discordant Cases

rpoB Mutation	Growth based DST	Treatment Regimen	Treatment Outcome
526Asn (AAC)	None	1 HRZE, MFX, CM 5 HRZ	Completed
	H, Z	2 HE, CM, CS PAS, MFX 1 RE, CM, CS, MFX **9 RE, MFX**	**Relapsed**
	H	1 RZE, CM, MFX 8 RZE	Completed
	H, ETO	2 HRZE **2 RZE**	**Failed**
	H	**12 RZE**	**Failed (Case 1)**
	H	**12 RZE, MFX**	**Relapsed (Case 3)**

CA Discordant Cases

rpoB Mutation	Growth based DST	Treatment Regimen	Treatment Outcome
516Phe (TTC)	H, Z	2 HRZE 19 E, MFX, LNZ, CM, CS, RFB	Cured
526Ser (TCC)	H, E, SM, ETO	2 RZ, MFX, CS, AMK 10 RZ, MFX	Completed
526Cys (TGC)	H, Z, E, SM, ETO, MFX, CIPRO, PAS	1 HRZE 1 AK, PAS, LNZ, MFX 5 AK, CS, PAS, LNZ, RFB, MFX 13 CS, PAS, LNZ, RFB	Cured

CA Discordant Cases

rpoB Mutation	Growth based DST	Treatment Regimen	Treatment Outcome
526Ser (AGC)	H	HRZE 11 RZE, LFX	Completed
	H, E	1 E, LFX, LNZ, CS, AMK 5 E, LFX, LNZ, CS, SM 3 E, LFX, LNZ, CS 9 ETO, LFX, CS	Completed
533Pro (CCG)	H, Z, E	1 HRZE 3 R, AMK, CS, PAS, LNZ, MFX 12 R, CS, PAS, MFX 2 R, CS, PAS, MFX	Completed
	H, SM, LFX, Clofaz, OFX, CIPRO	14 RE	Moved

Conclusions

- We think discordant mutations are rare
- Discordant mutations seem to have clinical importance
- Consider expanded regimens for patients with a discordant mutation and INH resistance
- Currently, we are treating patients with discordant mutation and INH resistance with MDR regimen
- Still have a lot to learn about molecular testing
- Keep your clinical hat on

Case 2

- **Initiated a MDR regimen**
  - PZA, EMB, AK, LFX, ETA, LNZ
  - Completed 3 months of AK
  - Close to completion of therapy
- **Repeat MRI stable**
- **Clinically no recurrence to date**

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