

Review of TB Drug Susceptibility: Molecules, Media, Challenges, and Opportunities

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Rifampin: does MGIT[®] miss resistance?

- Early validation studies show good correlation of MGIT rifampin results with those obtained by agar proportion method or BACTEC 12B
 - Horne. 2013. Metanalysis: JCM 51:393. Pooled sensitivity of MGIT 960 in detecting rif resistance, compared with other reference methods: 98.2% (but in some studies the reference method was another broth culture technique, namely BACTEC 12B/460)

Reports of rifampin resistance missed in MGIT testing

- 2007 presentation by Armand Van Deun at IUATLD World Congress in Capetown
- Published 2009: JCM 47:3501
 - Strains from Bangladesh and South Africa with certain specific mutations in *rpoB* gene
 - Strains tested as resistant by LJ and agar proportion methods, susceptible by BACTEC 12B or MGIT
 - Many of these strains were associated with treatment failure

“Low level rifampin resistance”

Working definition for the purpose of this talk:

Presence of a mutation in *rpoB*, which causes a change in amino acid sequence, leading to an increase in MIC above that of the wild type, but <1 ug/ml in MGIT so that the culture will test as susceptible in MGIT

2008 study by California lab

- Concerned that laboratories may miss rifampin resistance, leading to treatment failure
 - Recurrent tuberculosis, mostly due to relapse with the same TB strain, occurs in CA regularly
 - TB control program identified 103 patients with recurrent TB
 - CA Microbial Diseases Lab does *rpoB* sequencing to see if these strains have mutations leading to the kind of low level resistance that is missed by MGIT
 - Sequencing finds no mutations assoc with low level RIF resistance; one patient has classic high level RIF resistance
- [Abstract U-042, ASM General Meeting, 2009]

Conclusions from CA 2008 study of recurrent cases

- Mutations associated with low level rifampin resistance are not a major cause of relapse in CA
- Study does not support a need for changing rifampin susceptibility testing methods
- Study of 103 recurrent TB cases is not powerful enough to show how often mutations causing low-level resistance occur, or what impact these mutations have on TB treatment in USA

Reports of treatment failure associated with “low level” resistance continue

- Williamson IJTL D 2012 16:216
- 3 New Zealand cases in which Cepheid GeneXpert detected *rpoB* mutation, but culture tested susceptible to rif in MGIT
- Treatment failed in these 3 cases (all were INH-resistant)
- Mutations 516 Tyr and 526 Leu were assoc. w/ rifampin MICs of 0.25 and 0.5 respectively

Aug. 2013 J. Clin. Micro. articles by Rigouts, et al., and Van Deun, et al.

- Rigouts study: strains from Congo and Bangladesh

Note: Rif concentration in LJ is 40 ug/ml

RpoB mutation	No. of strains	Resistant in LJ	Resistant in MGIT at 1 ug/ml	Resistant in MGIT at 0.5 ug/ml
511 Pro	6	6	0	1
516 Tyr	6	6	0	1
526 Asn	5	5	1	1
533 Pro	14	14	0	0

Van Deun, et al. 2013 J. Clin. Micro 51(8):2633

Studied treatment outcome for TB strains with *rpoB* mutations

Mutation	% treatment failure or relapse
531Leu	64%
526Tyr	61% High level resistance
516Val	80%
511Pro	71%
516Tyr	57% (4/7) Low level resistance
526Asn	40% (2/5)
533Pro	60%

Low vs. high level resistance: both associated w/treatment failure in Bangladesh and Congo!

Van Deun, et al. 2013 JCM 51:2633, cont'd

Low level resistance mutations were 13% of all *rpoB* mutations in Bangladesh, and 10.6% of all *rpoB* mutations in Congo

Startling findings:

***rpoB* mutations associated with treatment failure are common and test susceptible in MGIT!**

USA perspective

Mutation	# in Campbell study	% in Campbell study*	% Resist in LJ (Rigouts)	% Resist in LJ (van Deun)	% Resist in Agar (Campbell)	% Resist in MGIT (Rigouts)	% Resist in MGIT (Lin)	MIC values (Berrada)
531 Leu	101	63.5%	100%	95%	100%	100%	100%	>8 (x5)
526 Tyr	18	11.3%	100%	100%	100%	71%	100% (4/4)	>8
516 Val	11	6.9%	100%	100%	100%	100%	100% (3/3)	>8 or 8
526 Leu	5	3.1%	100%	100%	60% (3/5)	60% (6/10)	100% (2/2)	8, 4
526 Asn	4	2.5%	100%	50% (2/4)	0% (0/4)	20% (1/5)	0% (0/2)	0.125, 0.25
526 Asp	4	2.5%	100%	100%	100% (4/4)	73% (8/11)	100% (5/5)	>8 (x3)
533 Pro	3	1.9%	100%	90% (9/10)	33% (1/3)	0% (0/14)	0% (0/1)	0.5
516 Tyr	2	1.3%	100% (6/6)	88% (7/8)	100% (2/2)	100% (6/6)	No data	0.25 (2) 0.5 (1)
522 Gln	2	1.3%	100%	100% (8/8)	100% (2/2)	73% (8/11)	No data	No data
531 Trp	2	1.3%	100% (4/4)	100% (3/3)	100% (2/2)	100% (4/4)	No data	>8 (x2)
All others	5	3.1%						

*Campbell study (CDC): Antimicrob Agents Chemother 2011 55(5):2032 does not necessarily reflect frequency of mutations in USA

What to make of all these data

- Some *rpoB* mutations cause low-level rifampin resistance, which can be associated with treatment failure, but the culture from these patients will test rif susceptible in MGIT
- Currently there is insufficient data to justify change in rif susceptibility testing in MGIT
- When GeneXpert reports a mutation in *rpoB*, but the MGIT rifampin testing is susceptible, GeneXpert is not necessarily wrong
 - *rpoB* sequencing at CDC or CA lab is recommended

Rifampin issues, cont'd

- Greater use of GeneXpert MTB/RIF will increase discovery of low-level resistance -- (GeneXpert says resistant, MGIT says susceptible → DNA sequencing)
- Expanded use of GeneXpert is recommended (see poster/talk by Lisa Pascopella)
- When there is low level resistance, e.g. MGIT/Xpert discrepancy or sequencing, treatment regimen may need to be modified
 - Anecdotal evidence: rif may still make an important contribution to the treatment regimen

More information is needed

- Is the situation in the USA different from what it is in Congo, Bangladesh, or New Zealand, where low level resistance to rifampin appears to be common, and associated with Rx failure?
- **If** in these countries the quality of the rifampin drug is poorer than in USA, or patients miss rifampin doses due to lax monitoring or interrupted drug supply, these conditions **might** foster survival and transmission of low-level resistant strains

Gathering more information

- Increased use of GeneXpert, and conflicting results between Xpert (resistant) and MGIT (susceptible) may enable discovery of low level resistance in USA
- Treatment outcomes for patients with low level resistance need to be studied: what is association with treatment failure or relapse in USA?

Low level rifampin resistance recap

Category	Presence of rpoB mutation	Expected GeneXpert result	Resistant by agar proportion	Resistant by MGIT	Clinical expectation
Rifampin resistant	Yes	Rif resistant	Resistant	Resistant	Rifampin not useful
Low level resistance	Yes	Rif resistant	+/-	Susceptible	Rifampin activity reduced, but still may be useful. More research needed. Treatment may fail.
Susceptible	No	Rifampin susceptible	Susceptible	Susceptible	Reliable contribution of Rif to treatment regimen

Diaryquinoline TMC207 (bedaquiline)

- Approved by FDA for treatment of TB, late 2012
- Excitement about
 - Shortening TB treatment regimens, especially in combination with PZA
 - Treatment of MDR cases

Bedaquiline, cont'd

Mouse study, treatment regimen more effective with addition of Bedaquiline (TMC207):

Mice treated 6 mos INH, rif, PZA: 5/30 relapse

Mice treated 4 mos TMC207, INH, rif, PZA: 1/17 relapse

Human clinical trial with MDRTB patients: 48% converted to culture negative within 8 weeks

Grosset 2012 IJTLD 16:1005

Resistance to TMC207 (bedaquiline)

- Resistance linked to mutations in C ring of ATP synthase
 - Asp 28→Gly, Asp28→Ala, Leu59→Val, Glu61→Asp, Ala63→Pro, Ile66→Met
- ATP synthase mutations associated with increases in MIC:
 - WT 0.03 µg/ml
 - Mutant strains 0.25 to 4 µg/ml

Not all resistance to Bedaquiline is due to ATP synthase mutations

- Huitric 2010 Antimicrob Agents Chemother 54:1022
 - Drug-resistant strains were selected *in vitro*
 - Some resistant strains had no mutation in the ATP synthase F0 or F1 operon, indicating there must be other, alternative resistance mechanisms

Tuesday morning: presentation by Beverly Metchock on Bedaquiline

Challenges of testing PZA in MGIT

- If inoculum is too heavy, pH may be raised and drug activity will decrease (false resistance)
- If inoculum is too light, it may not grow because low pH medium does not grow *M. tb* well (false susceptibility)
- If culture being inoculated is old, metabolically inactive cells may have increased susceptibility to PZA

So hard to get it right!

Significance of PZA in context of emerging drug resistance

- PZA is an important component of treatment regimens with new drugs Bedaquiline and PA824
- New CDC study shows 38% of MDR strains in USA were PZA-resistant (Kuratova et al. 2013. Clin Infect Dis, Epub ahead of print)

Proposal for PZA

- Use fresh cultures for inoculation to avoid false susceptibility
- **Avoid over-inoculation to avoid false resistance**
- When a culture tests PZA monoresistant, repeat and investigate
 - Investigation—is it from an extrapulmonary site or other reason to suspect *M. bovis*? This would support resistance
- When repeat result becomes available:
 - Resistant: confirmed. Report resistance.
 - Susceptible: sequence *pncA* gene including promoter region, or perform pyrazinamidase assay

Progress in PZA susceptibility testing

- CDC lab continues to investigate the link between *pncA* sequence and resistance to PZA
 - *pncA* sequencing likely to become most reliable predictor of PZA resistance
- CDC lab also working on pyrazinamidase method from MGIT tube
- Wednesday morning: Roy Tu'ua from Missouri State Public Health Laboratory will give talk on effectiveness of using reduced inoculum in MGIT drug suscept. testing

MGIT users: are we reporting some false-susceptible ethambutol results?

- Could be. Some strains with *embB* mutations are resistant in BACTEC 12B and agar proportion, but susceptible in MGIT
- Performance evaluation failures suggest trend of false EMB susceptibility in MGIT.
- Where possible, repeat EMB testing when INH resistance is found (agar proportion or *embB* sequencing)