Using MICs to Individualize Treatment for Patients with TB Disease

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Objectives

- Differentiate between Minimal Inhibitory Concentration (MIC) and Critical Concentration (CC) for antimicrobial susceptibility testing of for antimicrobial susceptibility testing of *Mycobacterium tuberculosis* and apply this knowledge when interpreting test results to determine the most appropriate treatment options
- Discuss the advantages of using MIC testing in individualized patient care and treatment to achieve favorable clinical outcomes for patients with drug resistant TB disease

Initial Presentation

- 49 y.o. female nurse, immigrated from Moldova ~1 y ago
- Aggressive cervical CA with lymph node metastases
- 4/2019 completed cisplatin/radiation therapy, good response
- Started adjuvant chemo, followed with serial CT scans
- CT 7/16/2019: Increased RUL nodule compared to 3/19
- FNA and core biopsy of lung nodule:
  - **Necrotizing granulomatous inflammation**
  - **Numerous AFB seen; MTB PCR positive**

Initial Evaluation Treatment

- Adjuvant chemotherapy was held
- Referred to local health department
- Patient clinically asymptomatic
- HIV-negative
- No prior TB or LTBI treatment
- Sputum AFB smear and NAAT negative, culture pending

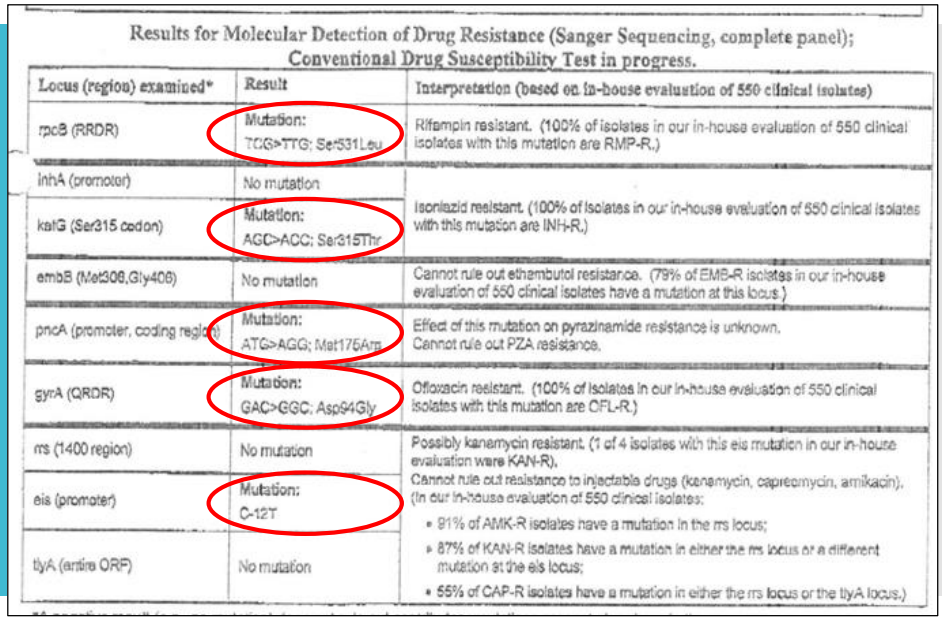
- 8/6/2019 Patient **started on RIPE**

Drug Susceptibility Results

- 8/15/19 Biopsy specimen grew AFB in culture, identified as **MTB by molecular probe**
- Isolate on LJ slant sent to jurisdictional public health lab
- 9/5/19 GeneXpert® **MTB detected, RIF resistance detected**
- 9/5/19 Isolate growth sent to CDC for MDDR* and to the Florida State Public Health Laboratory for HAINs/ and sequencing
- 9/9/19 RIPE stopped

*MDDR=Molecular Detection of Drug Resistance
- https://www.cdc.gov/tb/topic/laboratory/default.htm
- https://www.cdc.gov/tb/topic/laboratory/MDDRsubmissionform.pdf

CDC MDDR Report



Results for Molecular Detection of Drug Resistance (Sanger Sequencing, complete panel); 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	
katG (Ser315 codon)	Mutation: AGC>ACC; Ser315Thr	Isoniazid resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are INH-R.)
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 locus.)
pncA (promoter, coding region)	Mutation: ATG>AGG; Met176Arg	Effect of this mutation on pyrazinamide resistance is unknown. Cannot rule out PZA resistance.
gyrA (QRDR)	Mutation: GAC>GGC; Asp94Gly	Ofloxacin resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are OFL-R.)
rrs (1400 region)	No mutation	Possibly kanamycin resistant. (1 of 4 isolates with this eis mutation in our in-house evaluation were KAN-R). 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 a different mutation at the eis locus; • 55% of CAP-R isolates have a mutation in either the rrs locus or the tlyA locus.)
eis (promoter)	Mutation: C-12T	
tlyA (entire ORF)	No mutation	

Sequencing on MTB isolate performed at Florida DOH BPHL

	Target	Mutation	Detected by	Predicted (date)
	rpoB (RRDR)	Ser531Leu, TCG/TTG	MTBDRplus, DNA Seq.	**RIF-R, RFB-R**
	katG (ORF, aa 225-345)	Ser315Thr, AGC/ACC	MTBDRplus, DNA seq	**INH-R**
	mabA-inhA promoter	No mutation	MTBDRplus, DNA seq.	
	pncA (promoter, ORF)	Met175Arg, ATG/AGG	DNA seq.	**Possibly PZA-R**
	embB (ORF, aa 288-509)	No mutation	DNA seq.	Likely EMB-S
	gyrA/gyrB (QRDR, aa 1-132)	Asp94Gly, GAC,GGC	MTBDRsl DNA seq.	**Possibly FQ-R**
	rrs (1400 region)	No mutation	MTBDRsl	*Likely S to injectable drugs (ami, vio)
	eis (promoter)	WT band missing	MTBDRsl	***Likely R to injectable drugs (kan)**
Research use only	*atpE* (ORF)	No mutation	DNA seq.	*Resistance to bedaquiline is not predicted but cannot be ruled out.
Research use only	RV0678/mmpR (ORF)	No mutation	DNA seq.	*resistance to bedaquiline is not predicted but cannot be ruled out.
Research use only	*rplC* (ORF, aa 84-217)	No mutation	DNA seq.	*Resistance to linezolid is not predicted but cannot be ruled out.
Research use only	*rrl* (nt: 2191-2929)	No mutation	DNA seq.	*Resistance to linezolid is not predicted but cannot be ruled out.

***Determination of MTBC Drug susceptibilities by culture growth (phenotypic) methods is the gold standard**

Choosing Treatment for XDR-TB

- Asymptomatic
- Focal, pauci-bacillary TB disease (single nodule)
- Sputum: AFB smear, NAAT, and culture negative
- By molecular DST, patient has **XDR-TB**

Growth-based DST Results from State Public Health Lab

Specimen Source:	Unknown	Report S
Analyte/Assay		Result
TB Culture*		Acid Fast Bacilli Found
TB Drug Susceptibility		
Isoniazid 0.1		Resistant - Preliminary
Isoniazid 0.4		Resistant - Preliminary
Pyrazinamide		Resistant - Preliminary
Rifampin 1.0		Resistant - Preliminary
Ethambutol		Sensitive - Final

Phenotypic DST Results based on Critical Concentration

MTBC Agar Proportion Susceptibility*	% Resistant	Interpretation
Isoniazid 0.2 µg/mL	100 %	Resistant
Isoniazid 1.0 µg/mL	100 %	Resistant
Isoniazid 5.0 µg/mL	100 %	Resistant
Rifampin 1.0 µg/mL	100 %	Resistant
Ethambutol 5.0 µg/mL	0 %	Susceptible
Streptomycin 2.0 µg/mL	100 %	Resistant
Streptomycin 10.0 µg/mL	100 %	Resistant
Rifabutin 2.0 µg/mL	50 %	Resistant
Ciprofloxacin 2.0 µg/mL	100 %	Resistant
Kanamycin 5.0 µg/mL†	0 %	Susceptible
Ethionamide 10.0 µg/mL	50 %	Resistant
Capreomycin 10.0 µg/mL	100 %	Resistant
PAS 2.0 µg/mL	0 %	Susceptible
Ofloxacin 2.0 µg/mL	100 %	Resistant
Amikacin 4.0 µg/mL	0 %	Susceptible

Comments and Disclaimers
* See Report Comments and Disclaimers
* Susceptibility testing method: Indirect agar proportion, 7H10 medium. Resistance is defined as >1% (growth on drug-containing medium compared to drug-free medium).

MTBC Pyrazinamide Susceptibility*	Result
Pyrazinamide 100 µg/mL	Resistant

Comments and Disclaimers
* Susceptibility testing method: Mycobacteria Growth Indicator Tube (MGIT)

How can MICs inform the clinical treatment decision?

- Can RIF or RFB be increased to overcome resistance?
- Is high dose INH a possibility?
- Are all injectables equally resistant?
- Can FQ resistance be overcome?
- What is the susceptibility for BDQ?
- Based on level of LZD resistance can a safer, lower dose be used and still effectively kill MTB?

Growth-based Methods for Detection of Drug Resistance

What is the difference?

Critical Concentration:

The lowest concentration of drug that inhibits 95% of wild type strains that have never been exposed to anti-TB drugs (naïve strains)

Minimum inhibitory concentration:

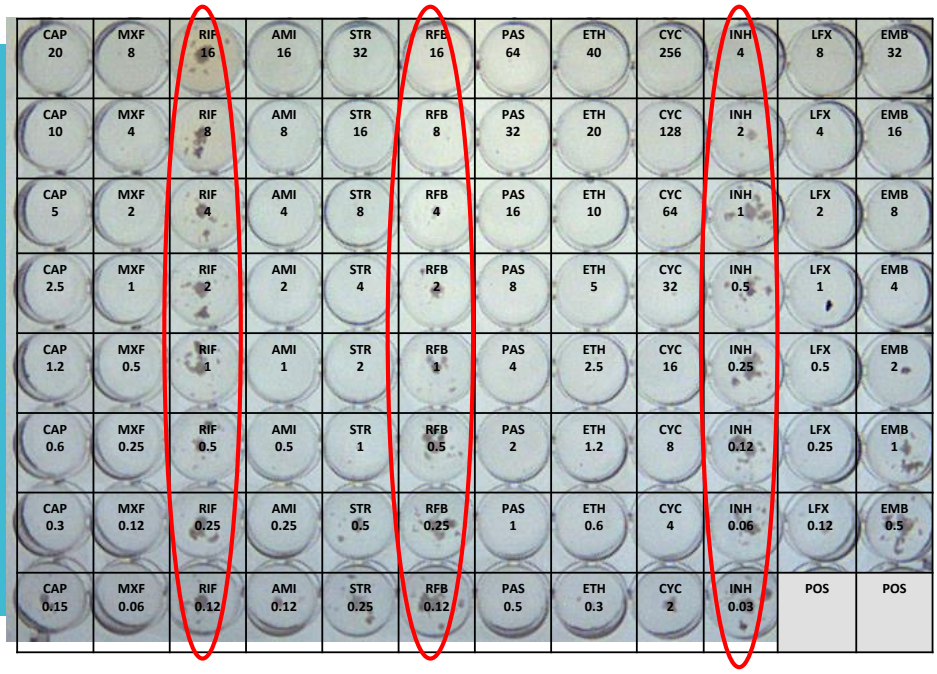
The lowest concentration of a drug in a series of dilutions that will inhibit the visible growth of microorganism

Growth-based Detection of Drug Resistance

BACTEC MGIT

Sensititre MIC

MIC Methods of Detection of Drug Resistance



What are the Advantages of MIC Method?

- More information for determination of susceptibility
- Tailored drug dosing, specific to patient:
  - Use lower dose if drug is toxic
  - Use higher drug dose above the MIC
  - Use drugs with a narrow therapeutic window
- Level of phenotypic susceptibility can be compared to genotypic susceptibility (e.g. low-level RIF resistance)
- Even if MIC breakpoints are not established, level of inhibition can be interpreted clinically
- MICs enable community surveillance of DST over time
- MIC plates can test 12 different drugs and be customized

What are the Challenges with the MIC method?

- Not an FDA-authorized method and must verify as a Laboratory Developed Test
- No established breakpoint for certain drugs/no manufacturer guidance
- Discordance between genotypic and phenotypic methods, or between two different phenotypic methods
- Longer turnaround time

Initial XDR-TB Therapy

- 10/7/2019: Patient initiated **BDQ, LZD, CS, CFZ, EMB+ MFX**
- Close laboratory and clinical monitoring, drug levels
- Tolerated regimen well
- With patient input, decided to treat patient with **BPaL***

*FDA-approved for extensively drug resistant or treatment intolerant TB disease

- https://www.tballiance.org/access/pretomanid-and-bpal-regimen
- https://www.fda.gov/media/128001/download

Florida DOH BPHL MIC Results

Isoniazid MIC	2 µg/mL		
Isoniazid Interpretation	**Resistant**	H	Susceptible: <0.25 Intermediate:0.25-1. 0 Resistant: ≥2
Rifampin MIC	>16 µg/mL		
Rifampin Interpretation	**Resistant**	H	Susceptible:≤1 Resistant: ≥2
Ethambutol MIC	2 µg/mL	Before RIPE given	
Ethambutol Interpretation	**Susceptible**		Susceptible: ≤2 Intermediate: 4.0 Resistant:≥ 8
Kanamycin Interpretation	**Not Tested**		Susceptible: ≤2.5 Resistant: ≥5
Rifabutin MIC	0.25 µg/mL		
Rifabutin Interpretation	**Susceptible**		Susceptible: ≤0.25 Resistant: ≥0.5
Ofloxacin Interpretation	**Not Tested**		Susceptible: ≤1 Resistant: ≥2
Ethionamide Interpretation	**Not Tested**		Susceptible: ≤1.2 Resistant: ≥2.5

Florida DOH BPHL MIC Results

Amikacin MIC	0.25 µg/mL	
Amikacin Interpretation	Susceptible	Susceptible: ≤2 Resistant: Breakpoint not established
Moxifloxacin MIC	8 µg/mL H	
Moxifloxacin Interpretation	No Interpretation	Susceptible: ≤0.12 Resistant: Breakpoint not established
Para-Aminosalicylic Acid MIC	<0.5 µg/mL	
Para-Aminosalicylic Acid Interpretation	Susceptible	Susceptible: ≤0.5 Resistant:Breakpoint not established
Cycloserine MIC	8 µg/mL	
Cycloserine Interpretation	Susceptible	Susceptible: ≤8.0 Resistant: Breakpoint not established
Capreomycin MIC	10 µg/mL H	
Capreomycin Interpretation	No Interpretation	Susceptible: ≤5 Resistant: Breakpoint not established

Florida DOH BPHL MIC Results

Levofloxacin MIC	8 µg/mL H	
Levofloxacin Interpretation	No Interpretation	Susceptible: ≤0.5 Resistant: Breakpoint not established
Linezolid MIC	0.12 µg/mL	
Linezolid Interpretation	Susceptible	Susceptible: ≤1.0; Resistant: Breakpoint not established.

Table 1. Molecular susceptibility sequencing results and therapeutic drug monitoring data

Drug (dose)	Trough (mcg/mL)	2h post-dose (mcg/mL)	6h post-dose (mcg/mL)	Typical peak serum concentration
Bedaquiline (200mg MWF)	0.51 (42.25h post dose)	1.40	1.42	1.2-1.8 (5-6h post dose, maintenance phase)
N-monodesmethyl Bedaquiline (metabolite)	0.22 (42.25h post dose)	0.24	0.27	
Pretomanid (200mg daily)	2.07 (18.25h post dose)	3.43	2.98	2.3 - 4.3 (5-6h post dose, at steady state)
Linezolid (600mg daily)	7.62 (18.25h post dose)	24.15 MIC = 0.12	17.88	12-26
Linezolid (600mg MWF)	<2.00*	19.04	13.6	12-26

*Trough sample was not collected, but based on the apparent elimination half-life, the linezolid concentration at 48 hours was calculated to be <2 mcg/ml, a value associated with minimal toxicity.

Therapeutic Drug Monitoring Laboratories

- University of Florida Infectious Diseases Pharmacokinetic Laboratory https://idpl.pharmacy.ufl.edu/formsand-catalog/
- National Jewish Medical Center https://www.nationaljewish.org/for-professionals/diagnostic-testing/adx/our-laboratories/therapeutic-drug-monitoring

Patient Follow Up

- Completed 6 months of BpaL
- Doing well a year after treatment completion
- Likely cured…..

Questions??