

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)
rpmB (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 (Met308,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; Met175Arg	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 CFL-R.)
rs (1400 region)	No mutation	Possibly kanamycin resistant. (1 of 4 isolates with this eis mutation in our in-house evaluation were KAN-R).
eis (promoter)	Mutation: C-12T	Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 550 clinical isolates:
tyA (antisense ORF)	No mutation	<ul style="list-style-type: none"> • 81% of AMK-R isolates have a mutation in the rs locus; • 87% of KAN-R isolates have a mutation in either the rs locus or a different mutation at the eis locus; • 55% of CAP-R isolates have a mutation in either the rs locus or the tyA locus.)

Sequencing on MTB isolate performed at Florida DOH BPHL

Target	Mutation	Detected by	Predicted (date)
<i>rpoB</i> (RRDR)	Ser531Leu, TCG/TTG	MTBDRplus, DNA Seq.	RIF-R, RFB-R
<i>katG</i> (ORF, aa 225-345)	Ser315Thr, AGC/ACC	MTBDRplus, DNA seq	INH-R
<i>mabA-inhA</i> promoter	No mutation	MTBDRplus, DNA seq.	
<i>pncA</i> (promoter, ORF)	Met175Arg, ATG/AGG	DNA seq.	Possibly PZA-R
<i>embB</i> (ORF, aa 288-509)	No mutation	DNA seq.	Likely EMB-S
<i>gyrA/gyrB</i> (QRDR, aa 1-132)	Asp94Gly, GAC,GGC	MTBDRsl DNA seq.	Possibly FQ-R
<i>rrs</i> (1400 region)	No mutation	MTBDRsl	*Likely S to injectable drugs (ami, vio)
<i>eis</i> (promoter)	WT band missing	MTBDRsl	*Likely R to injectable drugs (kan)
<i>atpE</i> (ORF)	No mutation	DNA seq.	*Resistance to <u>bedaquiline</u> is not predicted but cannot be ruled out.
RVo678/mmpR (ORF)	No mutation	DNA seq.	*resistance to <u>bedaquiline</u> is not predicted but cannot be ruled out.
<i>rplC</i> (ORF, aa 84-217)	No mutation	DNA seq.	*Resistance to <u>linezolid</u> is not predicted but cannot be ruled out.
<i>rrl</i> (nt: 2191-2929)	No mutation	DNA seq.	*Resistance to <u>linezolid</u> is not predicted but cannot be ruled out.
*Determination of MTBC Drug susceptibilities by culture growth (phenotypic) methods is the gold standard			

Research use only

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
<u>Analyte/Assay</u>		<u>Result</u>
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
<u>Rifampin 1.0 µg/mL</u>	100 %	Resistant
Ethambutol 5.0 µg/mL	0 %	Susceptible
Streptomycin 2.0 µg/mL	100 %	Resistant
Streptomycin 10.0 µg/mL	100 %	Resistant
<u>Rifabutin 2.0 µg/mL</u>	50 %	Resistant
<u>Ciprofloxacin 2.0 µg/mL</u>	100 %	Resistant
Kanamycin 5.0 µg/mL†	0 %	Susceptible
<u>Ethionamide 10.0 µg/mL</u>	50 %	Resistant
<u>Capreomycin 10.0 µg/mL</u>	100 %	Resistant
PAS 2.0 µg/mL	0 %	Susceptible
<u>Ofloxacin 2.0 µg/mL</u>	100 %	Resistant
<u>Amikacin 4.0 µg/mL</u> ;	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



**Sensitre
MIC**

MIC Methods of Detection of Drug Resistance

CAP 20	MXF 8	RIF 16	AMI 16	STR 32	RFB 16	PAS 64	ETH 40	CYC 256	INH 4	LFX 8	EMB 32
CAP 10	MXF 4	RIF 8	AMI 8	STR 16	RFB 8	PAS 32	ETH 20	CYC 128	INH 2	LFX 4	EMB 16
CAP 5	MXF 2	RIF 4	AMI 4	STR 8	RFB 4	PAS 16	ETH 10	CYC 64	INH 1	LFX 2	EMB 8
CAP 2.5	MXF 1	RIF 2	AMI 2	STR 4	RFB 2	PAS 8	ETH 5	CYC 32	INH 0.5	LFX 1	EMB 4
CAP 1.2	MXF 0.5	RIF 1	AMI 1	STR 2	RFB 1	PAS 4	ETH 2.5	CYC 16	INH 0.25	LFX 0.5	EMB 2
CAP 0.6	MXF 0.25	RIF 0.5	AMI 0.5	STR 1	RFB 0.5	PAS 2	ETH 1.2	CYC 8	INH 0.12	LFX 0.25	EMB 1
CAP 0.3	MXF 0.12	RIF 0.25	AMI 0.25	STR 0.5	RFB 0.25	PAS 1	ETH 0.6	CYC 4	INH 0.06	LFX 0.12	EMB 0.5
CAP 0.15	MXF 0.06	RIF 0.12	AMI 0.12	STR 0.25	RFB 0.12	PAS 0.5	ETH 0.3	CYC 2	INH 0.03	POS	POS

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
Isoniazid Interpretation

2 µg/mL
Resistant H

Susceptible: <0.25
Intermediate: 0.25-1.0
Resistant: ≥2

Rifampin MIC
Rifampin Interpretation

>16 µg/mL
Resistant H

Susceptible: ≤1
Resistant: ≥2

Ethambutol MIC
Ethambutol Interpretation

2 µg/mL
Susceptible

Before RIPE given

Susceptible: ≤2
Intermediate: 4.0
Resistant: ≥ 8
Susceptible: ≤2.5
Resistant: ≥5

Kanamycin Interpretation

Not Tested

Rifabutin MIC
Rifabutin Interpretation

0.25 µg/mL
Susceptible

Susceptible: ≤0.25
Resistant: ≥0.5
Susceptible: ≤1
Resistant: ≥2

Ofloxacin Interpretation

Not Tested

Ethionamide Interpretation

Not Tested

Susceptible: ≤1.2
Resistant: ≥2.5

Florida DOH BPHL MIC Results

Amikacin MIC	<u>0.25 µg/mL</u>	
Amikacin Interpretation	Susceptible	Susceptible: ≤2 Resistant: Breakpoint not established
Moxifloxacin MIC	<u>8 µg/mL</u>	
Moxifloxacin Interpretation	No Interpretation ^H	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	
Capreomycin Interpretation	No Interpretation ^H	Susceptible: ≤5 Resistant: Breakpoint not established

Florida DOH BPHL MIC Results

Levofloxacin MIC
Levofloxacin Interpretation

8 µg/mL H
No Interpretation

Susceptible: ≤0.5
Resistant:
Breakpoint not
established

Linezolid MIC
Linezolid Interpretation

0.12 µg/mL
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/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??