Clinical application of quantitative susceptibility testing

(if and when to use MICs)

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Infectious Diseases and International Health

No disclosures
How can quantitative susceptibility impact care at the bedside?
Outline

• Introduction to quantitative susceptibility testing

• The limited role of minimum inhibitory concentration (MIC) testing for fully drug susceptible TB

• The importance of MIC for drug-resistant TB or patients slow-to-respond given new data on individual pharmacokinetic variability

• Advantages/ disadvantages for TB/MDR-TB endemic areas

• Moving from resistance breakpoints: do we need an “intermediate” range?
**Principles of the 1% proportion method**

- INH 0.1 µg/ml + *M. tb* inoculum
- RIF 1.0 µg/ml + *M. tb* inoculum
- *M. tb* inoculum but no drug
- >1% of colonies compared to no drug = resistance

Single critical concentration with qualitative yes/no resistance (*different than most other infectious diseases*)

But *M. tb* is different → susceptibility testing on only subpopulation of organism in rapid growth phase, regimens used are 4+ different drugs

Crit concn can vary by media

But some isolates may teeter on Sus/Res, even using same media, same day of prep

Media prep at multiple concn for different drugs necessary for true MIC may be tedious, lack reproducibility
Minimum inhibitory concentrations—historically used in specialized settings on solid agar

<table>
<thead>
<tr>
<th>Drug</th>
<th>Susceptible</th>
<th>Moderately susceptible</th>
<th>Moderately resistant</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isoniazid</strong></td>
<td>≤0.1</td>
<td>0.2-1.0</td>
<td>2.0</td>
<td>≥4.0</td>
</tr>
<tr>
<td>MIC µg/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rifampin</strong></td>
<td>≤0.5</td>
<td>1.0-4.0</td>
<td>8.0</td>
<td>≥16.0</td>
</tr>
<tr>
<td>MIC µg/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


In practice this is uncommon

We’ll return to this concept

Majority with *rpoB* mutation
Now commercial microplate platform available

Lyophilized drug in prefilled wells, shelf-life 2 years at room temperature

Sensititre MYCOTB, TREK
- 122 *M. tb* isolates
- APM on 7H10
- **94%-100%** categorical agreement using Plate concn nearest to APM crit concn
- **Very few** resistant isolates by APM: Eg. Moxi 2 (1.6%), Amik 8 (6.5%)

### TABLE 1 Comparison of the APM critical concentrations and MycoTB plate ranges

<table>
<thead>
<tr>
<th>Agent</th>
<th>APM critical concn(s) tested (µg/ml)</th>
<th>MycoTB plate range (µg/ml)</th>
<th>MycoTB plate concn(s) nearest to the APM critical concn(s) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-line agents</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td>5.0, 10.0</td>
<td>0.5–32</td>
<td>4.0, 8.0</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.2, 1.0</td>
<td>0.03–4</td>
<td>0.25, 1.0</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1.0</td>
<td>0.12–16</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Second-line agents</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>5.0</td>
<td>0.12–16</td>
<td>4.0</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>25.0</td>
<td>2.0–256</td>
<td>32.0</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>5.0</td>
<td>0.3–40</td>
<td>5.0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>5.0</td>
<td>0.6–40</td>
<td>5.0</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>2.0</td>
<td>0.06–8.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2.0</td>
<td>0.25–32</td>
<td>2.0</td>
</tr>
<tr>
<td><em>p</em>-Aminosalicylic acid</td>
<td>2.0</td>
<td>0.5–64</td>
<td>2.0</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>0.5</td>
<td>0.12–16</td>
<td>0.5</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.0, 10.0</td>
<td>0.25–32</td>
<td>2.0, 8.0</td>
</tr>
</tbody>
</table>
But the real advantage is not in another yes/no qualitative resistance test...

I want to know if an isolate is:

1. **borderline susceptible**
   and I can maximize pharmacokinetics, particularly in a slow responder or...

2. **borderline resistant**
   if the drug options are limited (complex MDR/XDR-TB)
Majority of slow responders in Virginia had low $C_{2hr}$ levels of isoniazid (INH) and rifampin (RMP)

These are the patients where a borderline susceptible MIC would matter most

Heysell et al, *Emerg Infect Dis* 2010
Plasma TB Drug Activity (TDA) assay:

In BACTEC MGIT tubes quantifiable killing measured as time-to-detection (TTD), accurate and reproducible as colony counting.

- Control: eg. TTD = 120 hrs
- Experimental: eg. TTD = 240 hrs

$\frac{240\text{hr}}{120\text{hr}} = 2$

TB Drug Activity (TDA):

$\sim 1 \times 10^5$ CFU/ml

$50 \mu l$

$50 \mu l$

Plasma or serum

$M. tb$ bacilli

Incubate 72 hrs

MGIT fluorescence when CFU threshold reached
The TB drug activity assay is a metric for Cmax/ MIC

- Isoniazid
  - **R²=0.36**
  - **p=0.04**
  - **R²=0.38**
  - **p=0.03**
  - **R²=0.52**
  - **p=0.008**
  - **R²=0.88**
  - **P<0.001**

- Rifampin
  - **R²=0.36**
  - **p=0.04**
Poor plasma TB drug activity (Cmax/MIC) led to worse outcomes in Tanzania

Among subjects with the lowest TDA (≤1.5), only 2 (40%) were cured at 6 months compared to 10 (91%) with the higher TDA values (p=0.06)

Patient on moxifloxacin... is this even the correct breakpoint?
### Significant regional variation of MIC, and target concentration/MIC

#### TABLE 4 PTA expectation values, ofloxacin pharmacokinetic study in patients with MDR-TB, Cape Town and Durban, South Africa

<table>
<thead>
<tr>
<th>Ofloxacin daily dose (mg)</th>
<th>Overall PTA expectation</th>
<th>Cape Town PTA expectation</th>
<th>Durban PTA expectation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{AUC}/MIC \geq 100$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>0.45</td>
<td>0.33</td>
<td>0.65</td>
</tr>
<tr>
<td>1,000</td>
<td>0.57</td>
<td>0.46</td>
<td>0.76</td>
</tr>
<tr>
<td>1,200</td>
<td>0.66</td>
<td>0.57</td>
<td>0.83</td>
</tr>
<tr>
<td>1,400</td>
<td>0.73</td>
<td>0.64</td>
<td>0.89</td>
</tr>
<tr>
<td>1,600</td>
<td>0.77</td>
<td>0.70</td>
<td>0.91</td>
</tr>
<tr>
<td>$f_{AUC}/MIC \geq 40$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>0.83</td>
<td>0.77</td>
<td>0.94</td>
</tr>
<tr>
<td>1,000</td>
<td>0.87</td>
<td>0.83</td>
<td>0.95</td>
</tr>
<tr>
<td>1,200</td>
<td>0.90</td>
<td>0.87</td>
<td>0.96</td>
</tr>
<tr>
<td>1,400</td>
<td>0.92</td>
<td>0.89</td>
<td>0.97</td>
</tr>
<tr>
<td>1,600</td>
<td>0.93</td>
<td>0.91</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*With MIC of 2.0 µg/ml (WHO critical concentration), no patient achieved target $\geq 100$

PTA: probability of target attainment

In a TB endemic setting, Tanzania, MDR-TB patients (N=25) had a wide range of drug concentration/MIC.

<table>
<thead>
<tr>
<th>Drug (expected $C_{2hr}$ range)</th>
<th>$C_{2hr}$ µg/ml Mean ±SD</th>
<th>N below expected $C_{2hr}$ range (% total N)</th>
<th>MIC µg/ml Median (IQR)</th>
<th>$C_{2hr}$ /MIC Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Levofloxacin</strong> (8-12 µg/ml)</td>
<td>8.0 ±2.8</td>
<td>13 (52)</td>
<td>0.75 (0.25-1.0)</td>
<td>15.8 ±14.1</td>
</tr>
<tr>
<td><strong>Kanamycin</strong> (25-35 µg/ml)</td>
<td>26.0 ±10.2</td>
<td>10 (40)</td>
<td>1.2 (0.6-2.5)</td>
<td>22.9 ±18.7</td>
</tr>
<tr>
<td><strong>Ethionamide</strong> (1-5 µg/ml)</td>
<td>3.6 ±1.8</td>
<td>1 (4)</td>
<td>2.5 (1.2-5.0)</td>
<td>1.8 ±1.5</td>
</tr>
<tr>
<td><strong>Cycloserine</strong> (20-35 µg/ml)</td>
<td>33.9 ±12.2</td>
<td>3 (13)$^a$</td>
<td>8.0 (8.0-16.0)</td>
<td>4.3 ±3.0</td>
</tr>
<tr>
<td><strong>Pyrazinamide</strong> (20-60 µg/ml)</td>
<td>43.1 ±9.7</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

$^a$ Drugs concentration dependent in activity (like rifampin and isoniazid)
**Distribution of probable changes based on MIC, for MDR-TB patients (N= 13)**

<table>
<thead>
<tr>
<th>Modification</th>
<th>Frequency (%N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethionamide <em>change</em> to para-aminosalicylic acid</td>
<td>7 (54)</td>
</tr>
<tr>
<td>Ofloxacin or levofloxacin <em>change</em> to high-dose levofloxacin</td>
<td>6 (46)</td>
</tr>
<tr>
<td>Kanamycin <em>change</em> to amikacin</td>
<td>3 (23)</td>
</tr>
<tr>
<td>Amikacin or kanamycin <em>empirc change</em> to capreomycin</td>
<td>3 (23)</td>
</tr>
<tr>
<td>Amikacin <em>change</em> to kanamycin</td>
<td>1 (8)</td>
</tr>
</tbody>
</table>

**MIC can inform/alter the standardized MDR-TB regimen in Tanzania, even within a limited formulary**

*Mpgamama et al, submitted*
Even the best qualitative methods will be discrepant: 86 *M. tb* isolates (80% MDR) from Bangladesh

The MIC plate (TREK), using breakpoints, was the least discrepant when compared to other genotypic and phenotypic methods

Banu et al, in prep
Conclusions

- Commercial microplate MIC is available (and advantageous for many settings inexperienced in second-line DST) but use is limited for fully drug-susceptible TB—unless patient is slow-to-respond and drug concentrations can be measured and/or dose increased

- Given significant individual pharmacokinetic variability, including for MDR-TB drugs (fluroquinolones, aminoglycosides, ethionamide), MIC best applied with drug concentration measurement

- In the absence of drug concentration measurement (HPLC), MIC may still inform and alter MDR-TB management within a WHO formulary

- Quantitative susceptibility invites “borderline” or “intermediate” ranges but must be studied prospectively on a consistent platform (and informed by drug concentration/MIC targets)
Thank You

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Drug levels correct easily after first dose adjustment

INH daily
- Initial: 300 mg
- Follow-up: 450 mg
- Mean change: 4.0 μg/mL (SE=0.9)
  - p=0.01

INH biweekly
- Initial: 900 mg
- Follow-up: 1200 mg
- Mean change: 11.8 μg/mL (SE=3.1)
  - p=0.03

RMP daily/biweekly
- Initial: 600 mg
- Follow-up: 900 mg
- Mean change: 11.0 μg/mL (SE=2.4)
  - p<0.001

Heysell et al, Emerg Infect Dis 2010
The epidemiologic cut-offs (95%) could be wildly different and miss the subtlety of drug concentration/MIC.