The role of MIC determinations and *M. tuberculosis* subpopulations - what would be clinically useful?

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MIC determinations – why?

- Therapeutic drug monitoring (TDM)
- Low level resistance
- Establish drug susceptibility breakpoints
Individualized treatment

- MIC (susceptibility level)
- Resistance mutations

Host Immune defence

Clinical evaluation - TB-score
- Patient factors

Drug concentrations
- LC-MS/MS

Bacterial load – CFU/time to positivity (TTP)
TDM/MIC in clinical practice – Rifampicin

MIC distribution of rifampicin (8 studies, n=409)

Rifampicin drug concentrations (3 studies, n=238)

Host immunity represented by whole blood bactericidal assay (WBA)

Case 1. Drug susceptible pulmonary TB (600mg Rif)
MIC: 0.032 mg/L, Rif serum conc: 12.7 mg/L WBA -0.24, TB-score I: 2

**MIC distribution of rifampicin**

**Rifampicin drug concentrations**

**Host immunity represented by whole blood bactericidal assay (WBA)**

**TB-score I**

- Rifampicin-10mg/kg
- Rifampicin-high dose (20-35mg/kg)

- Sweden
- Ethiopia
Case 2. Drug susceptible pulmonary TB (600mg RIF)

MIC: 0.5 mg/L, Rif conc: 3.1 mg/L, WBA: 0.32 CFU/day, TB-scoreII:8

1. The RIF dose needed for cure is dependent on the MIC
2. Could higher RIF dosing vs MIC lead to shorter regimens?

Host immunity represented by whole blood bactericidal assay (WBA)

## MIC and Low level resistance

<table>
<thead>
<tr>
<th>Action</th>
<th>Mutation</th>
<th>MIC range</th>
<th>DST</th>
<th>Hain</th>
<th>Frequency (%)</th>
<th>Clinical outcome data</th>
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</thead>
<tbody>
<tr>
<td>HD FQ?</td>
<td><strong>FQ</strong>&lt;br&gt;<strong>gyrA A90V</strong></td>
<td>LEV 0.5-2&lt;br&gt;MOX 0.25-2</td>
<td>S/R(I)</td>
<td><strong>gyrA</strong>&lt;br&gt;Mut 1</td>
<td>20-30</td>
<td>+/-</td>
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<tr>
<td>HD AMI/CAP?</td>
<td>**AK/CAP: **&lt;br&gt;<strong>Eis C-12T, C-14T, G-10A</strong></td>
<td>KAN 8-32&lt;br&gt;AMI 1-4&lt;br&gt;CAP 2-8</td>
<td>KAN R&lt;br&gt;AMI S/R(I)&lt;br&gt;CAP S/R(I)</td>
<td><strong>Eis</strong>&lt;br&gt;Mut 1</td>
<td>5-22</td>
<td>-</td>
</tr>
<tr>
<td>HD INH?</td>
<td><strong>INH: inhA C-15T&lt;br&gt;A-16G&lt;br&gt;T-8C/A</strong></td>
<td>INH 0.25-2</td>
<td>INH R(I)</td>
<td><strong>inhA</strong>&lt;br&gt;Mut1&lt;br&gt;Mut2&lt;br&gt;Mut3ab</td>
<td>10-20</td>
<td>+/-</td>
</tr>
<tr>
<td>HD RIF?</td>
<td><strong>RIF: rpoB D516Y&lt;br&gt;L533P&lt;br&gt;H526S/L</strong></td>
<td>RIF:0.25-8</td>
<td>RIF S/R(I)</td>
<td><strong>rpoB</strong>&lt;br&gt;WT3/4&lt;br&gt;WT8&lt;br&gt;WT7-</td>
<td>&lt;0.1</td>
<td>+/-</td>
</tr>
<tr>
<td>HD RIB?</td>
<td><strong>RIF: rpoB D516V</strong></td>
<td>RIF:2-64</td>
<td>RIF R&lt;br&gt;RIB S/R(I)</td>
<td><strong>rpoB</strong>&lt;br&gt;Mut 1</td>
<td>5-32</td>
<td>+/-</td>
</tr>
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Low level resistance – mutations vs MICs

• Clinical outcome data limited
  – *inhA*, *gyrA* codon 90, *rpoB* A516V, *eis*, *embB* 306
  – Intermediate (I) - dose increase - TDM
• Each resistance mutation - MIC distribution
  – MIC determination if “low level resistance mutation”
• Are critical concentrations = clinical breakpoints = gold standard?
Pre-XDR or not?

MIC distribution of levofloxacin (13 studies, n=1274)

MDR; OFL R, LEV S, MOX S
Dose 750mgx1
Cmax: 11.2 mg/L
LEV MIC 1mg/L, OFL MIC 4mg/L
HAIN \textit{gyrA} mut 1 (A90V), WGS:A90V

MICs and clinical breakpoints

• Can we be sure about the gold standard?
  – EUCAST: PK/PD, clinical outcome, MIC
  – FQs, CAP, RIB, ETH/PTO, EMB?

• Methodology – MIC determination
  – No reference standard (!)
  – Categorical S/R validation of new methods (?)
    • LJ->7H10->B460->MGIT->TREK->…
  – MIC distributions for EUCAST

• Quality control
Ethambutol

Schön T et al JAC 2009
and unpublished observations

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<tr>
<td></td>
<td>LJ</td>
<td>7H10</td>
<td>7H10</td>
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<tr>
<td>EMB</td>
<td>2</td>
<td>5</td>
<td>5/10</td>
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Rifabutin (RIB S, RIF R ?)
Standard media for MIC testing of *M. tuberculosis*

- Löwenstein Jensen (LJ)
  - Pro: Widely used, inexpensive
  - Con: Variation in preparation, takes time (4v)
- Middlebrook 7H9 (used in TREK/MGIT)
  - Pro: 96-well format possible
  - Con: Contamination, ”readability” (TREK)
- Middlebrook 7H10/7H11
  - Pro: Used by CLSI (standardized protocol)
  - Con: Variation in preparation, contamination

WHO, ECDC, CLSI guidelines.
ECOFFs vs Clinical breakpoints

• ECOFF (epidemiological cut-off):
  – Highest MIC of organisms lacking phenotypically expressed resistance
  – The lowest possible breakpoint
  – A tool in determining clinical breakpoints
  – Sensitive detection/surveillance of resistance

• Clinical breakpoints:
  – MIC-concentrations decided by man to separate treatable from non-treatable organisms
  – Based on ECOFFs, PK/PD- and clinical outcome data
  – Predict outcome (SIR-system)
What is a wild type distribution?

- EUCAST: Gaussian MIC distribution for organisms without resistance mechanisms
- Establishing a clinical breakpoint (SIR)
  - Wild-type MIC distributions (ECOFF)
  - PK/PD simulations
  - Clinical outcome data
- ECOFF is **NOT** = clinical breakpoint
  ...nor the critical concentration:

  “…the lowest concentration of drug that will inhibit 95% (90% for pyrazinamide) of wild strains of M. tuberculosis that have never been exposed to drugs, while at the same time not inhibiting clinical strains of M. tuberculosis that are considered to be resistant (e.g. from patients who are not responding to therapy)”.

MIC distributions for EUCAST

- Literature study
  - Truncation and non-standard dilutions
- http://mic.eucast.org/Eucast2
- Standard methods (7H9/10/11, LJ, MGIT)
  - Not colorimetric methods, QC
- Compared to other pathogens
  - Bias towards inclusion of MDR/XDR isolates
Rifampicin MIC-distribution

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>WHO (2013/1998)</th>
<th>CLSI (2011)</th>
<th>WHO MGIT</th>
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<tbody>
<tr>
<td>RIF</td>
<td>LJ 40</td>
<td>7H10 1</td>
<td>7H10 1</td>
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<td></td>
<td></td>
<td></td>
<td>MGIT 1</td>
</tr>
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Quality control in MIC determination

• Reference MIC-ranges for H37Rv
  – Used for QC in DST of bacterial pathogens
  – Reproducibility – NOT S/R only!

• Proficiency testing
  – Not only S/R
  – H37Rv and isolates close to Bp (MOX/OFL, EMB, RIF)
What is the reproducibility of your comparator DST method?

- ISO 20776-2: within ± 1 dilution of the mode for ≥95% of the results
- *Usually below* ± 1 dilution

Fluconazole MIC distributions of 34 individual *Candida glabrata* isolates (black bars) compared with the MICs obtained by 51 repeated tests of a single *C. glabrata* isolate (striped bars) originally determined to have a MIC of 2 μg/ml.
Summary – MIC determinations

• TDM - quantify resistance
• Phenotype – genotype correlation
• Establishing clinical breakpoints