Current strategies for MIC determination and setting of clinical breakpoints

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The reference method

2003 20 june DIN Berlin
CEN TC140/WG10

2004 22 april DIN Berlin
Combined meeting with
ISO ISO/TC 212 WG4
Vienna Agreement

2005 Vote on first draft and comments
by all Member Countries

2006 Final version 27 October 2006,
8th CEN, 6th ISO meeting
ISO 20776-1

2007 Final version validation ISO 20776-2.

11/4/2019 Rotterdam, Optimised dosing of antibiotics
Minimum inhibitory concentration (MIC)

The reference method: microdilution

Measure of potency of the antibacterial effect

<table>
<thead>
<tr>
<th>Antibiotic Concentration (mg/L)</th>
<th>Mueller Hinton</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

Bacterial suspension: Inoculum 5 (2-8) $10^5$ cfu/ml

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Minimum inhibitory concentration (MIC)

2-fold increasing antibiotic concentrations in mg/L in Mueller Hinton

Bacterial suspension: Inoculum 5 (2-8) \(10^5\) cfu/ml

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<th>Antibiotic Concentration (mg/L)</th>
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</thead>
<tbody>
<tr>
<td>Incubation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Incubate 36 +/- 1º C</td>
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</tr>
<tr>
<td>18 +/- 2 hours</td>
<td></td>
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</tr>
</tbody>
</table>

After incubation: MIC = Lowest concentration with no **visible** growth

MIC = 2 mg/L

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Minimum inhibitory concentration (MIC)

Number of bacteria in this tube varies between 0-10^8 CFU/ml

What happened in the tubes?

Bacterial growth
Bacterial kill
Continuous process over time

\[
\frac{dN}{dt} = \left( \lambda \cdot \left(1 - \frac{N}{N_{\text{max}}} \right) - k \cdot \frac{C^\gamma}{C^\gamma + EC_{50}^\gamma} \right) \cdot N
\]

MIC is the result of these processes over time

Mouton et al 1997

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“The” MIC

Does NOT Quantify bacterial growth

Does NOT Quantify bacterial kill

It is the result of these biological processes over time

High variability and is not very reproducible

The use of other methods

• All methods need to be validated versus the reference method
• algorithm based on a few measurements.
• Not 2-fold dilution.
• Totally different approach
• repetitive turbidimetric monitoring of bacterial growth during an abbreviated incubation period.

* 2-fold dilution,
* micro-dilution,
* growth or no-growth (turbidometric and colorimetric (oxidation-reduction indicator) growth detection).

* different inoculum from the reference method
Gradiënt-tests

- Increasing concentration on the strip
- Antibiotic diffuses into the agar

Ceftolozane/Tazobactam (C/T 256)

Disc diffusion

- Do not result in a value in mg/L, but in mm of the zone.
Variation in measurements

- Biological variation within one strain
- Between-strain variation
- Between-laboratory variation

Disc diffusion *S. aureus* and cefoxitin

Different areas
~26000 observations (strains)
13 sources (different labs)
Different time periods
Susceptible strains: 22-35mm
Variation in measurements

- Biological variation within one strain
- Between-strain variation
- Between-laboratory variation

S aureus ATCC 29213 in 1 laboratory

1 strain
122 measurements
6 months
10 labtechnicians
Zones between 24-31 mm

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www.EUCAST.org
Compare the two distributions

~26000 strains

1 strain

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Second example on the variability

*S aureus* and linezolid MIC determined by gradient test (Etest®)

Analysis:
22 different strains
5 different laboratories
Sent in quadruplicate (blind fashion)
440 observations

Mouton, 2018, p2374

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MIC-distribution of the strains

440 observations in 5 labs
→ 20 observations per strain

Mouton, 2018, p2374
Linezolid and *S. aureus*

Source of the variation

<table>
<thead>
<tr>
<th>Observations (n)</th>
<th>Sum of squares (% of total error)</th>
<th>Explained</th>
<th>R²</th>
<th>Unexplained assay variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>440</td>
<td>227.82 (100%)</td>
<td>109.22 (47.9%)</td>
<td>23.57 (10.3%)</td>
</tr>
</tbody>
</table>

**Epidemiological cut-off (ECOFF)**

ECOFF: 0.5 mg/L

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Mouton, 2018, p2374

[www.eucast.org](http://www.eucast.org)
Conclusion MIC determination

• Considerable amount of variation
• The only conclusion that can be drawn is, whether the bacteria is within the wild-type distribution or not.
• Do not use such values in individual patient care

<table>
<thead>
<tr>
<th>MIC found</th>
<th>Interpretation for target attainment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within WT, ≤ECOFF</td>
<td>ECOFF</td>
</tr>
<tr>
<td>&gt;ECOFF</td>
<td>MIC + two 2-fold dilutions^6</td>
</tr>
</tbody>
</table>

^6Number of dilutions could be higher or lower than two depending on the proficiency of the lab and the drug—species distribution.

Mouton, 2018, p564

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Literature..... example

Be careful about MICs to amoxicillin for patients with Streptococci-related infective endocarditis.

In multivariate analysis, the only factor associated with in-hospital mortality was MIC for amoxicillin between 0.25 and 2mg/L (p=0.04; OR= 2.23 [1.03–4.88]) whereas protective factor was performance of cardiac surgery for IE (p=0.001, OR = 0.23 [0.1–0.56]).

• Population level, not individual level

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Setting clinical breakpoints... why?

Report an advise to the clinic

• Is there a high probability that the therapy will work or not?
Many factors involved

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MIC distribution in breakpoint setting

- Wild-type distribution is needed
- The results of the interpretation needs to be reproducible, therefore the chosen value will not split the wild-type distribution

Many factors involved

- Target micro-organism
- MIC distribution (ECOFF)
- PK/PD indices
- Required exposure
- Clinical breakpoint
- PK model for a population + MCS
- Pharmacokinetics
- Dosing regimen
- Concentrations required at specific sites, example urine, CSF
- Clinical indication
PK/PD indices - exposure

- How much exposure of the antibiotic to the bug is needed to achieve antibacterial effect?

- For example:
  - 40% $fT>MIC$ or 60% $fT>MIC$
  - Or $AUC/MIC$

Many factors involved

- Target micro-organism
- MIC distribution (ECOFF)
- Clinical indication
- Concentrations required at specific sites, example urine, CSF
- Clinical breakpoint
- Required exposure
- PK/PD indices
- PK model for a population + MCS
- Pharmacokinetics
- Dosing regimen
Dosing regimen and PK model

- What are the dosing regimen currently used?
- What is the pharmacokinetics of this antibiotic in human?
- What are the concentrations reached in humans?
- Perform Monte Carlo simulation with a population model representing the average patient with different dosing regimen.
  - The target needs to be reached in 95-99% of the population (of average patients, not ICU patients)

Many factors involved

- Target micro-organism
- MIC distribution (ECOFF)
- Clinical indication
- Concentrations required at specific sites, example urine, CSF
- Clinical breakpoint
- Required exposure
- PK/PD indices
- PK model for a population + MCS
- Pharmacokinetics
- Dosing regimen
Site specific breakpoints?

- There are some site specific breakpoints:
  - Concentrations reached in CSF are much lower compared to urine

**Streptococcus pneumoniae**

- Penicillins

<table>
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<tr>
<th>MIC breakpoints (mg/L)</th>
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</thead>
<tbody>
<tr>
<td>S ≤</td>
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</table>

<table>
<thead>
<tr>
<th>Benzylpenicillin (indications other than meningitis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
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Ceftazidime en *Pseudomonas aeruginosa*

- MIC

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PK/PD indices

<table>
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<tr>
<th>5. Pharmacodynamics</th>
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<tbody>
<tr>
<td>%fT&gt;MIC for bacteriostasis</td>
</tr>
<tr>
<td>%fT&gt;MIC for 2 log reduction</td>
</tr>
<tr>
<td>%fT&gt;MIC from clinical data</td>
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PK and MCS

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Setting the breakpoint - an example

- MIC 8mg/L

Setting the breakpoint - an example

- MIC 8mg/L
- Target $f_T>MIC$ 45-65%
Setting the breakpoint - an example

- MIC 8mg/L
- Target $f_T$ > MIC 45-65%
  - For Pseudomonas 3 times daily 1000mg is not enough.
  - The breakpoint cannot be <8 because of the WT distribution

Breakpoint is 8 mg/L with the following instruction:

Breakpoints to report

- These breakpoints are used to report to the clinicians
  - S: "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.
  - R: Resistant: A microorganism is categorised as "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure.
  - I: Susceptible, increased exposure: A microorganism is categorised as "Susceptible, Increased exposure" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.

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Many factors involved

Target micro-organism

MIC distribution (ECOFF)

Required exposure

PK/PD indices

Clinical breakpoint

Clinical indication

Concentrations required at specific sites, example: urine, CSF

PK model for a population + MCS

Pharmacokinetics

Dosing regimen

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