EUCAST
Establishing disk diffusion zone diameter breakpoints

Examples to explain the species related presentations soon available on the EUCAST website.

Contact Gunnar.Kahlemeter@ltkronoberg.se
MIC distributions in zone diameter histograms
Linezolid

S.aureus  E.faecalis  S.pneumoniae

Top: Clinical isolates from 25 labs

ATCC strain for each pathogen

Inhibition zone (mm)

No. of observations

0 10 20 30 40

2 mg/L 1 mg/L 0.5 mg/L 0.25 mg/L

0.25 mg/L  0.5 mg/L  1 mg/L  2 mg/L
MIC distributions in zone diameter histograms
Linezolid

No. of observations

Linezolid / Staphylococcus aureus
Linezolid / Enterococcus faecalis
Linezolid / Streptococcus pneumoniae

MIC distributions include isolates from multiple sources, geographical areas, and time periods. Data are for the entire range of dilutions and cannot be used to infer dilutions at 0.25 mg/L.

S. aureus

E. faecalis

S. pneumoniae

Inhibition zone (mm)

20 22 24 26 28 30 32 34 35

20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

0.25 mg/L 0.5 mg/L 1 mg/L 2 mg/L
From the EUCAST website (link below). The graph shows the distribution zone diameters (X-axis) for consecutive clinical isolates of *E. faecalis* for the EUCAST gentamicin 10 disk microgram disk; and the correlation between zone diameters (X-axis) and MIC-values (Y-axis) on a separate collection of strains with (n=74) and without high level aminoglycoside resistance. In the EUCAST distribution program the latter correlation is shown when the user clicks on the "MIC/Zone correlation"-button below graph.

http://217.70.33.99/Eucast2/SearchController/regShow.jsp?id=29873&showIsolateOnGrapf=true
P. aeruginosa /PTZ 30-6 ug
82 non-consecutive isolates chosen because of resistance

Breakpoints

<table>
<thead>
<tr>
<th>Breakpoints</th>
<th>S</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC</td>
<td>≤16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Zone diameter</td>
<td>≥19</td>
<td>&lt;19</td>
</tr>
</tbody>
</table>
P. aeruginosa /PTZ 30-6 ug

Piperacillin-tazobactam / Pseudomonas aeruginosa
EUCAST zone diameter distribution - Reference database 2011-05-27
EUCAST disk diffusion method

Distributions include culled data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance.

Breakpoints

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<thead>
<tr>
<th>MIC</th>
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<tr>
<td>≤16</td>
<td>&gt;16</td>
<td></td>
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<td>≥19</td>
<td></td>
<td>&lt;19</td>
</tr>
</tbody>
</table>

Epidemiological cut-off: WT ≥ 19 mm (MIC: ≤ 16 mg/L)
Clinical breakpoints: S ≥ 19 mm, R < 19 mm

738 observations (2 data sources)
E. coli /Ceftazidime 10 ug
203 non-consecutive isolates chosen because of resistance

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</tr>
</thead>
<tbody>
<tr>
<td>MIC</td>
<td>$\leq 1$</td>
<td>$&gt; 4$</td>
</tr>
<tr>
<td>Zone diameter</td>
<td>$\geq 22$</td>
<td>$&lt; 19$</td>
</tr>
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</table>
E. coli / Ceftazidime 10 ug

Ceftazidime / Escherichia coli
EUCAST zone diameter distribution - Reference database 2011-05-27
EUCAST disk diffusion method

Breakpoints

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<th>MIC</th>
<th>S</th>
<th>R</th>
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<tr>
<td>≤1</td>
<td>≥22</td>
<td>&lt;19</td>
</tr>
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</table>

No of isolates

- 512
- 256
- 128
- 64
- 32
- 16
- 8
- 4
- 2
- 1
- 0.5
- 0.25
- 0.125

Zone diameter (mm)
Distribution of zone diameters obtained through repeated testing of one ATCC-strain as part of daily internal quality control of methodology. Ten lab.technicians were involved over a period of 6 months. The distribution can be described by the blue curve which is transposed to the next few slides to demonstrate the magnitude of inherent methodological variation.

Cefoxitin / Staphylococcus aureus ATCC 29213
EUCAST zone diameter distribution - Reference database
EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance.

Disk content: 30
Epidemiological cut-off: WT ≥ 22 mm (MIC: -)

Clinical breakpoints: S ≥ 22 mm, R < 22 mm
Cefoxitin / Staphylococcus aureus MSSA

EUCAST zone diameter distributions - Reference database
EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance.

11 published studies:
1364 meca-negative S. aureus

Blue curve (see previous slide) describes the distribution obtained when one ATCC strain is tested daily for 6 months.
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E.coli v. mecillinam

- The following 4 slides show the correlation between zone diameters (X-axis) and MIC-values (see colour scheme).
- **Slide 1**: the complete material - 931 clinical *E.coli* isolates from uncomplicated UTI.
- **Slide 2**: subset of 274 isolates resistant to ampicillin (which in this case is equal to isolates belonging to the ampicillin non-wild type).
- **Slide 3**: subset of 657 isolates susceptible to ampicillin (which in this case is equal to isolates belonging to the ampicillin wild type).
- **Slide 4**: subset of 560 isolates susceptible to all other (except mecillinam) agents tested (n=16 + mecillinam).
E. coli vs. mecillinam
931 isolates from the ECOSENS II Study

E. coli from women with uncomplicated UTI: 180-200 from each of Greece, Portugal, Austria, UK and Sweden.
E. coli vs. mecillinam

274 isolates resistant to ampicillin

• Slide 2: subset of 274 isolates resistant to ampicillin (which in this case is equal to isolates belonging to the ampicillin non-wild type).
Slide 3: subset of 657 isolates susceptible to ampicillin (which in this case is equal to isolates belonging to the ampicillin wild type).

**E. coli vs. mecillinam**

657 isolates susceptible to ampicillin
Slide 4: subset of 560 isolates susceptible to all other (except mecillinam) agents tested (n=16 + mecillinam).

**E. coli vs. mecillinam**

560 isolates susceptible to all other 16 agents tested
Levofloxacin / Staphylococcus aureus

EUCAST MIC Distribution - Reference Database 2011-05-06

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

MIC
Epidemiological cut-off: WT ≤ 0.5 mg/L

Clinical breakpoints: S ≤ 1 mg/L, R > 2 mg/L

27556 observations (13 data sources)
**S. aureus: Levofloxacin MIC vs Disk**

*n=442*

\[ y = 13.2 - 0.21x \]

\[ r = 0.96 \]

Red arrow – inherent variation in MIC values for a given zone diameter.

Blue arrow – variation in zone diameters for a given MIC-value.

**Characteristics graph used by CLSI to correlate MIC to Zone diameter** (courtesy JMI laboratories and Ron Jones and Helio Sader)
WT = 4 MIC dilutions equals 4 x 3 mm in a zone diameter distribution
For most agents and species with few technical difficulties the wild type can be described by an MIC distribution over 3 – 5 dilution steps and a zone diameter distribution of 10 – 14 mm.

WT = 4 MIC dilutions equals 4 x 3 mm in a zone diameter distribution.