Educational Workshop

EW01: Antimicrobial susceptibility testing with EUCAST breakpoints and methods

Arranged with the European Committee on Antimicrobial Susceptibility Testing (EUCAST)

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Canton - What is new in 2013: update on EUCAST breakpoints

WHAT IS NEW IN 2013:
UPDATE ON EUCAST BREAKPOINTS

EUCAST General Committee (GC)
All European Countries + Countries from outside EUCAST
Steering Committee
BSAC, CA‐SFM, CRG, NWGA, SRGA
+ 3 reps from the GC ± 2 "visiting" members from the GC

Subcommittees
Antifungals
Anaerobes
Resistance mechanisms

EUCAST implementation

Q1
Canton - What is new in 2013: update on EUCAST breakpoints

- EUCAST General Committee
  - 35 countries, including Russia and Australia
  - New countries with interest of being represented (Brazil, US, Japan…)

- National breakpoint committees and NACs
  - Continuous consultation on breakpoints
  - New countries already represented in the General Committee formed a NAC (Portugal, Germany, …)
  - New countries outside Europe have declared interest to form a NAC (Brazil, US, …)

- Four new Standard Operation Procedures (03-01-2013)
  - 3.0 Review and revision of breakpoints
  - 4.0 EUCAST committees
  - 5.0 EUCAST interaction with NACs
  - 6.0 maintenance of EUCAST web site (on going)

National AST Committees (NACs), January 2013

Implementation of EUCAST breakpoints, January 2013
Canton - What is new in 2013: update on EUCAST breakpoints

Guidelines followed in EARS-Net EQA 2012
(National representation is biased, as in UK NEQAS)

<table>
<thead>
<tr>
<th>Year</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>22</td>
</tr>
<tr>
<td>2010</td>
<td>29</td>
</tr>
<tr>
<td>2011</td>
<td>47</td>
</tr>
<tr>
<td>2012</td>
<td>61</td>
</tr>
</tbody>
</table>

EUCAST implementation

Q2

EUCAST breakpoints, 2013
Canton - What is new in 2013: update on EUCAST breakpoints

**New version of EUCAST breakpoint tables v3.0 (Jan, 2013)**

- **New drugs**: Ceftaroline breakpoints
- **New microorganisms**: Pasteurella multocida, Campylobacter
- **Rewording of notes**: S. pneumoniae and penicillins, HLGR in Enterococcus spp.
- **New footnotes for**: Yersinia enterocolitica
- **New technical notes**: Specific reading instructions for correct interpretation of the disk diffusion tests - S. maltophilia and SXT - Staphylococci and benzypenicillin - Enterococci and vancomycin
- **New expressions of antimicrobials**: fosfomycin-trometamol, now fosfomycin oral - cefuroxime, now cefuroxime i.v. - cefuroxime axetil, now cefuroxime oral
- **New table of PK/PD (non-species related) breakpoints**

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**EUCAST breakpoints, 2013**

All new modifications highlighted in yellow

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**New version of EUCAST breakpoint tables v3.0 (Jan, 2013)**

**New drugs**: Ceftaroline breakpoints

Clinical breakpoints for ceftaroline have been set by EUCAST as follows:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>MIC breakpoint (mg/L)</th>
<th>Shear contact (W)</th>
<th>Zone diameter breakpoint (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>0.5</td>
<td>0.5</td>
<td>Note</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Streptococcus Group A, B, C, G</td>
<td>None</td>
<td>None</td>
<td>Note</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>0.25</td>
<td>0.25</td>
<td>Note</td>
</tr>
<tr>
<td>Enterococci in Europe</td>
<td>0.03</td>
<td>0.03</td>
<td>Note</td>
</tr>
<tr>
<td>PK/PD (non-species related) breakpoints</td>
<td>0.5</td>
<td>0.5</td>
<td>-</td>
</tr>
</tbody>
</table>

1. Refer susceptibility from susceptibility to benzylpenicillin.
2. Based on PK/PD target for Gram-negative organisms.
3. Disk diffusion breakpoints corresponding to the MIC breakpoints are currently being established.
4. The zone diameter breakpoint for S. aureus is based on the MIC distribution of MRE2A. For zone diameters 19-21 mm an MIC determination will confirm the susceptibility.
Canton - What is new in 2013: update on EUCAST breakpoints

New version of EUCAST breakpoint tables v3.0 (Jan, 2013)

- New drugs: Ceftaroline QC ranges

<table>
<thead>
<tr>
<th>Organisms</th>
<th>MIC (μg/L)</th>
<th>Disk content (μg)</th>
<th>Inhibition zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli ATCC 25922</td>
<td>0.06</td>
<td>0.03-0.12</td>
<td>18</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 29213</td>
<td>0.25</td>
<td>0.12-0.5</td>
<td>20</td>
</tr>
<tr>
<td>Staphylococcus pneumonia ATCC 49219</td>
<td>0.015</td>
<td>0.006-0.33</td>
<td>18</td>
</tr>
<tr>
<td>Haemophilus influenzae ATCC 8463</td>
<td>note</td>
<td>note</td>
<td>note</td>
</tr>
</tbody>
</table>

2. Control ranges are currently being established.

New version of EUCAST breakpoint tables v3.0 (Jan, 2013)

- New microorganisms - Pasteurella multocida

Clinical breakpoints based on ECOFFs in the absence of MIC-clinical correlations

Disk diffusion breakpoints calibrated with MIC breakpoints

New version of EUCAST breakpoint tables v3.0 (Jan, 2013)

- Clinical breakpoints based on ECOFFs in the absence of MIC-clinical correlations

Disk diffusion breakpoints calibrated with MIC breakpoints
New version of EUCAST breakpoint tables v3.0 (Jan, 2013)

- PK/PD breakpoints listed separately

PK/PD (non-species related) breakpoints

<table>
<thead>
<tr>
<th>MIC (mg/L) brpts*</th>
<th>≤2</th>
<th>R&gt;2 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone (mm) brpts*</td>
<td>≥22</td>
<td>R&lt;22 mm</td>
</tr>
</tbody>
</table>

Insufficient evidence
(Literature: "not enough evidence for a breakpoint" or "no indication")

IE
Can not be substituted
Can be supplemented with an MIC without interpretation.

Inappropriate drug
(Literature: poor drug - don’t use!

Can be substituted with an automatic “R”

*when numbers are the same = no intermediate category
Canton - What is new in 2013: update on EUCAST breakpoints

The EUCAST breakpoints philosophy

- MIC by broth microdilution is the current reference method (ISO)
- If MIC testing is used, it should either be the standard reference method as described in the ISO document or an MIC method shown to give equivalent result to the ISO method.
- If disk diffusion is used and validated following ISO guidelines, the categorization of S/I/R is fully equivalent to that of MIC testing.
- If there is a deviation in a local method (medium, incubation time, volume, inoculum, …) that local method should also be validated against the ISO doc. The same is true for commercial systems.
- Many labs state they use MICs but do deviate from the ISO protocol which may explain differences in the results.

The EUCAST breakpoints philosophy

- Disk diffusion calibrated with MIC values
EUCAST breakpoints: What is coming 2013-14?

**Ongoing breakpoints**
- New agents with EMA:
  - anti-MRSA cephalosporins
  - macrolides
  - anti-mycobacterials
  - glycopeptides
- Colistin (in conjunction with CLSI and under TATFAR initiative)
- Topical agents (if available based on systemic breakpoints, otherwise based on ECOFFs)
  - Fluoroquinolones and enterococci in UTI
  - Corynebacterium spp.
  - Co-amoxiclav and Enterobacteriaceae in UTI
  - Acinetobacter spp. and sulbactam
- Other organisms
  - Legionella spp., Pseudomonas non-aeruginosa spp.
  - Actinomyces, Nocardia, Streptomyces, HACEK
  - Aeromonas, Vibrio, Leuconostoc, Lactobacillus, Pedicoccus

Transatlantic Taskforce on Antimicrobial Resistance

- **Antimicrobial resistance is a significant and multifaceted public health problem**
- **Purpose of the taskforce**
  - To identify urgent antimicrobial resistance issues that could be better addressed by intensified cooperation between the US and the EU within the following key areas:
    1. Appropriate therapeutic use of antimicrobials in the medical and veterinary communities
    2. Prevention of healthcare- and community-associated drug-resistant infections
    3. Strategies for improving the pipeline of new antimicrobial drugs

Colistin / polymixin B breakpoints

- Initiative to set common EUCAST and CLSI breakpoints
- Memorandum of Understanding (MOU)
- Creation of ad hoc Working Group
  - 2 co-chairs, 2+2 members representing EUCAST and CLSI
  - absence of members with commercial / industry affiliation
  - colistin and polymixin B breakpoints for
    - Enterobacteriaceae
    - Pseudomonas aeruginosa
    - Acinetobacter spp.
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EUCAST breakpoints: What is coming 2013-14?

Breakpoints are based on epidemiological cut-off values (ECOFFs)

EUCAST General Committee

All European Countries + Countries from outside

EUCAST Steering Committee

BSAC, CA‐SFM, CRG, NWGA, SRGA

And 3 reps from the General Committee

Subcommittees

Antifungals

Expert Rules

Anaerobes

Resistance mechanisms

National Breakpoint Committees

F, N, NL, S, UK

Experts (ECDC Networks, ESCMID Study Groups)

EUCAST breakpoints: What is in coming 2013-14?

Proposed fluoroquinolone clinical breakpoints for Enterococcus spp. from UTI

General consultation until 14 June 2013

Antimicrobial agent | Clinical breakpoint for urinary tract infections | ECOFF
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>4 mg/L</td>
<td>4</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>4 mg/L</td>
<td>4</td>
</tr>
</tbody>
</table>

2013 NEWS

www.eucast.org
Canton - What is new in 2013: update on EUCAST breakpoints

www.eucast.org

EUCAST website changes

www.eucast.org

...all free, no charge!
Canton - What is new in 2013: update on EUCAST breakpoints
What’s new in EUCAST methods?

Derek Brown
EUCAST Scientific Secretary

MIC determination

- MH-F broth for broth for broth microdilution testing of fastidious microorganisms
- Gradient MIC testing on MH-F agar

EUCAST methodology for fastidious organisms

<table>
<thead>
<tr>
<th>Media</th>
<th>Disk diffusion</th>
<th>Broth microdilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MH-F broth</td>
<td>MH-F broth*</td>
<td>MH-F broth*</td>
</tr>
<tr>
<td>McFarland 0.5</td>
<td>5 x 10⁵ cfu/mL</td>
<td>5 x 10⁵ cfu/mL</td>
</tr>
<tr>
<td>Incubation</td>
<td>16-20 h</td>
<td>16-20 h</td>
</tr>
<tr>
<td>35 ± 1°C</td>
<td>35 ± 1°C</td>
<td>35 ± 1°C</td>
</tr>
<tr>
<td>5% CO₂</td>
<td>Ambient air (sealed panels)</td>
<td>Ambient air (sealed panels)</td>
</tr>
</tbody>
</table>

* MH-F broth
M-H broth with 5% lysed horse blood and 20 mg/L β-NAD. Horse blood lysed by repeated freezing and thawing.
Brown - What is new in 2013: update on EUCAST methods

Validation of MH-F broth

- Comparison of distributions of MICs with MH-F broth with collated distributions on EUCAST MIC distribution website (http://mic.eucast.org/Eucast2/)

- Tests on *H. influenzae* and *S. pneumoniae*

- MH-F data courtesy of Ron Jones, JMI Laboratories, USA
Brown - What is new in 2013: update on EUCAST methods

Validation of MH-F broth
N. Influenzae with ciprofloxacin

EUCAST database
12688 observations
21 data sources

Broth microdilution with MH-F broth
150 isolates tested in duplicate

Validation of MH-F broth
S. pneumoniae with cefotaxime

EUCAST database
12800 observations
24 data sources

Broth microdilution with MH-F broth
100 isolates

Validation of MH-F broth
S. pneumoniae with trimethoprim-sulfamethoxazole

EUCAST database
31592 observations
11 data sources

Broth microdilution with MH-F broth
100 isolates
Brown - What is new in 2013: update on EUCAST methods

Gradient tests on MH-F medium

- Details of tests validated by manufacturer listed on EUCAST website
  http://www.eucast.org/antimicrobial_susceptibility_testing/compliance_of_manufacturers

- Compliance of manufacturers of AST materials and devices with EUCAST guidelines
  - Data are based on information from manufacturers
  - The accuracy of data in these tables is not verified by EUCAST and the inclusion of any materials or devices does not indicate endorsement by EUCAST.

Gradient tests: Etest

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Agents in EUCAST tables but not available</th>
<th>Validated for MH-F</th>
<th>(Inhibitor/antibiotic format)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td>Cefadroxil</td>
<td>Chloramphenicol</td>
<td>Amoxicillin–clavulanate (4)</td>
</tr>
<tr>
<td></td>
<td>Cefalexin</td>
<td>Clindamycin</td>
<td>Amoxicillin–clavulanate (2)</td>
</tr>
<tr>
<td></td>
<td>Cefazolin</td>
<td>Erythromycin</td>
<td>Amoxicillin–clavulanate</td>
</tr>
<tr>
<td></td>
<td>Ceftibuten</td>
<td>Levofoxacin</td>
<td>Amoxicillin–clavulanate</td>
</tr>
<tr>
<td></td>
<td>Roxithromycin</td>
<td>Moxifloxacin</td>
<td>Amoxicillin–clavulanate</td>
</tr>
</tbody>
</table>

Gradient tests: MIC Test Strip

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Agents in EUCAST tables but not available</th>
<th>Validated for MH-F</th>
<th>(Inhibitor/antibiotic format)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td>Cefadroxil</td>
<td>Chloramphenicol</td>
<td>Amoxicillin–clavulanate (4)</td>
</tr>
<tr>
<td></td>
<td>Cefalexin</td>
<td>Clindamycin</td>
<td>Amoxicillin–clavulanate (2)</td>
</tr>
<tr>
<td></td>
<td>Cefazolin</td>
<td>Erythromycin</td>
<td>Amoxicillin–clavulanate</td>
</tr>
<tr>
<td></td>
<td>Ceftibuten</td>
<td>Levofoxacin</td>
<td>Amoxicillin–clavulanate</td>
</tr>
<tr>
<td></td>
<td>Roxithromycin</td>
<td>Moxifloxacin</td>
<td>Amoxicillin–clavulanate</td>
</tr>
</tbody>
</table>

Compliance of manufacturers with EUCAST guidelines, 11 March 2013

Gradient tests: Etest

- The accuracy of data in these tables is not verified by EUCAST and the inclusion of any materials or devices does not indicate endorsement by EUCAST.

Gradient tests: MIC Test Strip

- The accuracy of data in these tables is not verified by EUCAST and the inclusion of any materials or devices does not indicate endorsement by EUCAST.
Compliance of manufacturers with EUCAST guidelines, 11 March 2013

Gradient tests: M.I.C.Evaluator

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Agents in EUCAST tables but not available</th>
<th>Validated for MIC-Evaluator</th>
<th>10% (similar to EUCAST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shandon</td>
<td>Amoxicillin</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Fisher</td>
<td>Ceftriaxone</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>Ciprofloxacin</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Oxoid</td>
<td>Ceftazidin</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>Azithromycin</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Oxoid</td>
<td>Moxifloxacin</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>Ticarcillin</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Oxoid</td>
<td>Ticarcillin-Clavulanic acid</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>Tobramycin</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Oxoid</td>
<td>Colistin</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>Daptomycin</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Oxoid</td>
<td>Stenotrophomonas maltophilia</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>Trimethoprim-Sulfamethoxazole</td>
<td>No</td>
<td>Fixed 2:1 ratio</td>
</tr>
</tbody>
</table>

Disk diffusion

- Reading instructions for difficult tests added to breakpoint table
- _Campylobacter_ method and zone diameter breakpoints
- _Pasteurella multocida_ method and zone diameter breakpoints
- Zone diameter breakpoints for new agents
- Data on correlation of disk diffusion breakpoints with MIC breakpoints
- Revision of zone diameter breakpoints
- Screening tests

Reading instructions for difficult disk diffusion tests in breakpoint tables

[http://www.eucast.org/antimicrobial_susceptibility_testing/breakpoints](http://www.eucast.org/antimicrobial_susceptibility_testing/breakpoints)

Stenotrophomonas maltophilia and trimethoprim-sulfamethoxazole

Outer zone ≥16mm = S  
No trace of zone = R
Brown - What is new in 2013: update on EUCAST methods

### Reading instructions for difficult disk diffusion tests in breakpoint tables

**S. aureus** and benzylpenicillin
- Zone diam $\geq 26$ mm
- Fuzzy zone edge = S
- Sharp zone edge = R

### Reading instructions for difficult disk diffusion tests in breakpoint tables

**Enterococcus.** spp. and vancomycin
- Zone diam $\geq 12$ mm
- Sharp zone edge = S
- Fuzzy zone edge = R

### EUCAST disk diffusion method for *Campylobacter* spp. (*jejuni* and *coli*)

<table>
<thead>
<tr>
<th>Media</th>
<th>MH-F (pre-dried plates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>McFarland 0.5</td>
</tr>
<tr>
<td>Incubation</td>
<td>Microaerobic environment 41°C 24 h*</td>
</tr>
<tr>
<td>Reading</td>
<td>EUCAST standard reading</td>
</tr>
</tbody>
</table>

* Campylobacter *coli* isolates with insufficient growth are reincubated immediately and read after a total of 40-48 h incubation
**Calibration of disk diffusion method for Campylobacter spp.**

- 30 *C. jejuni* and 27 *C. coli* from two collections
- MIC by ISO broth microdilution
- Disk diffusion in duplicate by EUCAST method
- Study in collaboration between EUCAST, Central Veterinary Institute (CVI), Lelystad, the Netherlands and National Institute for Health and Welfare (THL), Turku, Finland.

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Ciprofloxacin 5 µg vs. MIC, *Campylobacter jejuni* and *coli*

- 57 isolates tested in duplicate
- Breakpoints:
  - MIC: ≤0.5, >0.5 mg/L
  - Zone diameter: ≥26, <26 mm

---

Erythromycin 15 µg vs. MIC, *Campylobacter jejuni*

- 30 isolates tested in duplicate
- Breakpoints:
  - MIC: ≤4, >4 mg/L
  - Zone diameter: ≥20, <20 mm
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**Erythromycin 15 µg vs. MIC, Campylobacter coli**
27 isolates tested in duplicate

- **Breakpoints**
  - MIC: S ≤ 8, R > 8 mg/L
  - Zone diameter: S ≥ 24, R < 24 mm

- **ECOFF**
  - MIC WT ≤ 8 mg/L

**Tetracycline 30 µg vs. MIC, Campylobacter jejuni and coli**
29 isolates tested in duplicate

- **Breakpoints**
  - MIC: S ≤ 2, R > 2 mg/L
  - Zone diameter: S ≥ 30, R < 30 mm

- **ECOFF**
  - MIC WT ≤ 1 mg/L (C. jejuni), ≤ 2 mg/L (C. coli)

**EUCAST disk diffusion method for Pasteurella multocida**

- **Medium**: MH-F
- **Inoculum**: McFarland 0.5
- **Incubation**: 5% CO₂, 35±1°C, 18 h ± 2 h
- **Reading**: Read zone edges at the point of complete inhibition of growth viewed from the front of the plate with the lid removed and with reflected light
**Calibration of disk diffusion method for *Pasteurella multocida***

http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation

- 131 *P. multocida* from two collections
- MIC by gradient tests on MH-F agar
- Disk diffusion in duplicate by EUCAST method

- Study in collaboration between EUCAST, Central Veterinary Institute (CVI), Lelystad, the Netherlands and JMI Laboratories, North Liberty, Iowa, USA

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**Benzylpenicillin 1 unit vs. MIC**

*Pasteurella multocida*, 131 isolates

- **Breakpoints**
  - MIC: S≤0.5, R>0.5 mg/L
  - Zone diameter: S≥17, R<17 mm

- **ECOFF**
  - WT≤0.5 mg/L

---

**Amoxicillin-clavulanate 2-1 µg vs. MIC**

*Pasteurella multocida*, 128 isolates

- **Breakpoints**
  - MIC: S≤1, R>1 mg/L
  - Zone diameter: S≥15, R<15 mm

- **ECOFF**
  - WT≤1 mg/L
Brown - What is new in 2013: update on EUCAST methods

**Ciprofloxacin 5 µg vs. MIC**  
*Pasteurella multocida*, 131 isolates

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>ECOFF</th>
<th>Breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.06</td>
<td></td>
<td>≤0.06 mg/L</td>
</tr>
<tr>
<td>≤0.06</td>
<td></td>
<td>≤0.06 mg/L</td>
</tr>
<tr>
<td>≥27</td>
<td></td>
<td>≥27 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Nalidixic acid 30 µg vs. Ciprofloxacin MIC**  
*Pasteurella multocida*, 62 isolates

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>ECOFF</th>
<th>Breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.06</td>
<td></td>
<td>≤0.06 mg/L</td>
</tr>
<tr>
<td>≤0.06</td>
<td></td>
<td>≤0.06 mg/L</td>
</tr>
<tr>
<td>≥23</td>
<td></td>
<td>≥23 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Zone diameter breakpoints for new agents**

- MIC breakpoints set as part of the marketing authorisation through EMA
- 2012 Ceftaroline
Brown - What is new in 2013: update on EUCAST methods

Zone diameter breakpoints for ceftaroline

<table>
<thead>
<tr>
<th>Organisms</th>
<th>MIC breakpoints (mg/L)</th>
<th>Zone diameter breakpoints (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S ≤ R &gt; S ≥ R&lt;</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1 1</td>
<td>20 20</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>0.25 0.25</td>
<td>Note² Note²</td>
</tr>
<tr>
<td>Streptococcus Groups A, B, C, G</td>
<td>Note¹ Note¹ Note¹ Note¹</td>
<td></td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>0.03 0.03</td>
<td>IP IP</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>0.5 0.5</td>
<td>23 23</td>
</tr>
</tbody>
</table>

1. Infer susceptibility from susceptibility to benzylpenicillin
2. Screen with oxacillin disk
IP = In preparation

Zone diameter breakpoints for new agents - ceftaroline

Ceftaroline 5 µg vs. MIC
S. aureus, 100 clinical isolates tested at 2 sites x2

Note in breakpoint table: For isolates with zone diameters 19-21 mm, determine the MIC to confirm the susceptibility

Data on correlation of zone diameter breakpoints with EUCAST MIC breakpoints

Extensive new data added to EUCAST website

MIC-zone diameter distributions

Zone diameter distributions with MIC data identified in histograms
Brown - What is new in 2013: update on EUCAST methods

**MIC-zone diameter distributions**

http://mic.eucast.org/Eucast2

**Zone diameter distributions with MICs**

http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation

**Screening for beta-lactam resistance in S. pneumoniae**

Oxacillin screen can give useful information on susceptibility to beta-lactam agents other than benzylpenicillin

Detailed data on EUCAST website

http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation

Poster 1578, Sunday 13.30-14.30
Brown - What is new in 2013: update on EUCAST methods

**Oxacillin 1 µg vs. Benzylpenicillin MIC**

*Streptococcus pneumoniae*, 148 clinical isolates

- **Classical screen for beta-lactam non-susceptibility in S. pneumoniae**
- **CLSI and EUCAST**

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>No of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.008</td>
<td>6</td>
</tr>
<tr>
<td>≤ 0.015</td>
<td>2</td>
</tr>
<tr>
<td>≤ 0.03</td>
<td>6</td>
</tr>
<tr>
<td>≤ 0.06</td>
<td>16</td>
</tr>
<tr>
<td>≤ 0.12</td>
<td>4</td>
</tr>
<tr>
<td>≤ 0.25</td>
<td>2</td>
</tr>
<tr>
<td>≤ 0.5</td>
<td>2</td>
</tr>
<tr>
<td>≤ 1</td>
<td>2</td>
</tr>
<tr>
<td>≤ 2</td>
<td>2</td>
</tr>
<tr>
<td>≤ 4</td>
<td>2</td>
</tr>
<tr>
<td>≤ 8</td>
<td>2</td>
</tr>
<tr>
<td>≤ 16</td>
<td>2</td>
</tr>
<tr>
<td>≤ 32</td>
<td>2</td>
</tr>
</tbody>
</table>

**Breakpoints**

- Benzylpenicillin MIC: S ≤ 0.06, R > 2 mg/L
- Oxacillin zone diameter (screen): S ≥ 20, R < 20 mm

**ECOFF**

- WT ≤ 0.06 mg/L

**Oxacillin 1 µg vs. Ampicillin MIC**

*Streptococcus pneumoniae*, 153 clinical isolates

- **Isolates with oxacillin zone diameter ≥ 8 mm are susceptible to ampicillin**

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>No of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.008</td>
<td>6</td>
</tr>
<tr>
<td>≤ 0.015</td>
<td>2</td>
</tr>
<tr>
<td>≤ 0.03</td>
<td>6</td>
</tr>
<tr>
<td>≤ 0.06</td>
<td>16</td>
</tr>
<tr>
<td>≤ 0.12</td>
<td>4</td>
</tr>
<tr>
<td>≤ 0.25</td>
<td>2</td>
</tr>
<tr>
<td>≤ 0.5</td>
<td>2</td>
</tr>
<tr>
<td>≤ 1</td>
<td>2</td>
</tr>
<tr>
<td>≤ 2</td>
<td>2</td>
</tr>
<tr>
<td>≤ 4</td>
<td>2</td>
</tr>
<tr>
<td>≤ 8</td>
<td>2</td>
</tr>
<tr>
<td>≤ 16</td>
<td>2</td>
</tr>
<tr>
<td>≤ 32</td>
<td>2</td>
</tr>
</tbody>
</table>

**Breakpoints**

- Ampicillin MIC: S ≤ 0.5, R > 2 mg/L
- Oxacillin zone diameter (screen): Ampicillin S ≥ 8 mm

**Oxacillin 1 µg vs. Cefotaxime MIC**

*Streptococcus pneumoniae*, 147 clinical isolates

- **Isolates with oxacillin zone diameter ≥ 8 mm are susceptible to cefotaxime**

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>No of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.008</td>
<td>6</td>
</tr>
<tr>
<td>≤ 0.015</td>
<td>2</td>
</tr>
<tr>
<td>≤ 0.03</td>
<td>6</td>
</tr>
<tr>
<td>≤ 0.06</td>
<td>16</td>
</tr>
<tr>
<td>≤ 0.12</td>
<td>4</td>
</tr>
<tr>
<td>≤ 0.25</td>
<td>2</td>
</tr>
<tr>
<td>≤ 0.5</td>
<td>2</td>
</tr>
<tr>
<td>≤ 1</td>
<td>2</td>
</tr>
<tr>
<td>≤ 2</td>
<td>2</td>
</tr>
<tr>
<td>≤ 4</td>
<td>2</td>
</tr>
<tr>
<td>≤ 8</td>
<td>2</td>
</tr>
<tr>
<td>≤ 16</td>
<td>2</td>
</tr>
<tr>
<td>≤ 32</td>
<td>2</td>
</tr>
</tbody>
</table>

**Breakpoints**

- Cefotaxime MIC: S ≤ 0.5, R > 2 mg/L
- Oxacillin zone diameter (screen): Cefotaxime S ≥ 8 mm
### Screening for beta-lactam resistance in *S. pneumoniae*

<table>
<thead>
<tr>
<th>Oxacillin (μg disk) Zone diameter (mm)</th>
<th>Antimicrobial agent</th>
<th>Further testing and/or interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20 mm</td>
<td>All beta-lactam agents for which clinical breakpoints are listed, including those with &quot;Note&quot;</td>
<td>Report susceptible irrespective of clinical indication.</td>
</tr>
<tr>
<td>&lt; 20 mm*</td>
<td>Benzylpenicillin (meningitis) and thienamycins (all infections)</td>
<td>Test by an MIC method for the agent considered for clinical use and interpret according to the clinical breakpoints.</td>
</tr>
<tr>
<td></td>
<td>Benzylpenicillin (for infections other than meningitis)</td>
<td>Oxacillin zone diameter ≥ 8 mm: Report susceptible. Oxacillin zone diameter &lt; 8 mm: Determine the MIC of the beta-lactam agent intended for clinical use but for ampicillin, amoxicillin and piperacillin (without and with beta-lactamase inhibitor) infer susceptibility from the MIC of ampicillin.</td>
</tr>
<tr>
<td></td>
<td>Ampicillin and amoxicillin (without and with beta-lactamase inhibitor), ceftaxime, cephalosporins</td>
<td>Test by an MIC method for the agent considered for clinical use and interpret according to the clinical breakpoints.</td>
</tr>
<tr>
<td></td>
<td>Other beta-lactam agents</td>
<td>Test by an MIC method for the agent considered for clinical use and interpret according to the clinical breakpoints.</td>
</tr>
</tbody>
</table>

### Screening for beta-lactam resistance in *H. influenzae*

Benzylpenicillin screen can give useful information on susceptibility to beta-lactam agents

**Detailed data on EUCAST website**
http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation

---

**Benzylpenicillin 1 unit vs. β-lactamase**

*H. influenzae*, 148 clinical isolates

- **Breakpoints**
  - Benzylpenicillin zone diameter (screen): S≤12, R<12 mm

All beta-lactamase positive isolates give zone diameters <12 mm with benzylpenicillin 1 unit disk
Benzylpenicillin 1 unit vs. PBP mutations
H. influenzae, 104 β-lactamase negative clinical isolates

<table>
<thead>
<tr>
<th>Inhibition zone diameter (mm)</th>
<th>PBP mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Isolates with PBP mutations give zone diameters <12 mm with benzylpenicillin 1 unit disk

Screening for beta-lactam resistance in H. influenzae
Supplementary table

<table>
<thead>
<tr>
<th>Benzylpenicillin 1 unit disk, Zone diameter (mm)</th>
<th>Beta-lactamase</th>
<th>Further testing and/or interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 12 mm</td>
<td></td>
<td>Report susceptible to all beta-lactam agents for which clinical breakpoints are listed (including those with &quot;Note&quot;).</td>
</tr>
<tr>
<td>&lt; 12 mm</td>
<td>Beta-lactamase negative</td>
<td>Test susceptibility to the beta-lactam agent intended for clinical use.</td>
</tr>
<tr>
<td></td>
<td>Beta-lactamase positive</td>
<td>For ampicillin, amoxicillin and piperacillin, report resistant. For other beta-lactam agents, test susceptibility to the beta-lactam agent intended for clinical use.</td>
</tr>
</tbody>
</table>

Revision of zone diameter breakpoints

- Breakpoints may be revised in the light of new information and changes highlighted in breakpoint tables (http://www.eucast.org/clinical.breakpoints)
- e.g. Telithromycin and strep groups A,B,C,G
  - 2012 S ≥22 mm, R <19 mm
  - 2013 S ≥20 mm, R <17 mm
- e.g. Teicoplanin and S. pneumoniae
  - 2012 S ≥18 mm, R <18 mm
  - 2013 S ≥17 mm, R <17 mm
EUCAST methods
What is coming in 2013-14?

- MIC
  - Further validation of gradient tests
- Disk diffusion
  - Corynebacterium spp.
  - Pseudomonas non-aeruginosa
  - Neisseria gonorrhoeae
  - New agents authorised by EMA
  - Some rapidly-growing anaerobes
- Guidance note on Pseudomonas cepacia
ECOFFs
ECOFFs
ECOFFs
ECOFFs

MIC wild type distributions and epidemiological cut-off values

Gunnar Kahlmeter
EUCAST, ESCMID and ECDC
Clinical microbiology, Växjö, Sweden

Breakpoints

**Clinical breakpoints**
- MIC concentrations decided by man (based on clinical outcome data, MIC distributions, accepted dosing, PK/PD-data) to distinguish treatable from non-treatable organisms.
- should not divide wild type MIC distributions of target organisms.
- may render wild type organisms Susceptible (S), Intermediate (I) or Resistant (R)
- may change with a change in circumstances (indications, new resistance mechanisms, new dosing)
Breakpoints

ECOFF
- The ECOFF distinguishes between organisms without and with phenotypically expressed resistance mechanisms for a species and a drug in a defined test system.
- Within a species, it is the highest MIC for organisms lacking phenotypically expressed resistance.
- Organisms without resistance mechanisms are not by default treatable and organisms with resistance mechanisms are not by default resistant.
- In a species considered susceptible (S) to the agent, it is the lowest possible S breakpoint.

Establishing ECOFFs
- ECOFFs should only be determined on
  - MICs determined with methods calibrated to the internationally agreed standard method for broth microdilution (ISO)
  - large numbers of MICs (n>100, ideally >1000)
  - MICs from different places (minimum 3)
  - MICs performed by many investigators (minimum 3, ideally 10 or more)
Tentative rules for aggregating MIC distributions and determine ECOFF

<table>
<thead>
<tr>
<th>MIC distributions*</th>
<th>Distributions which disagree**</th>
<th>Action (none, aggregate or aggregate and determine ECOFF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>None***</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>Aggregate 3</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Aggregate 4 and determine ECOFF</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>Aggregate 3</td>
</tr>
<tr>
<td>7</td>
<td>&gt;1</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>Aggregate 5 and determine ECOFF</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>Aggregate 4</td>
</tr>
<tr>
<td>10</td>
<td>&gt;1</td>
<td>None</td>
</tr>
<tr>
<td>etc.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EGPN: Enterococcus faecium

Distribution of MICs and inhibition zone diameters defined by agent, species and test systems are freely available on the internet (www.eucast.org)
MIC distributions and ECOFFs on EUCAST website

- 25,000 MIC distributions
- Up to 100,000 MIC values per distribution
- Data from many investigators (1–100 per distrib.)
- Data from many time periods (1950–)
- Data from many geographical areas and projects (USA, Europe, Australia, Far East, South America, Sentry, Mystic, etc)
- Data of multiple origin (Human clinical data, Surveillance programs, Veterinarian data, Wildlife, Food safety programs)
- Database secure on three servers in different parts of Dusseldorf under the official responsibility of EUCAST and ESCMID.
- Ownership:
  - Software and administration: ECDC/ESCMID
  - Database: individual ownership of original data

Factors influencing the median and width of WT distributions
The **median** of the MIC distribution:

- The inherent susceptibility of the species to the drug
- Anything systematically influencing the activity of the drug:
  - Medium – variation in MICs depending on medium
  - Inoculum – increasing MICs with higher inocula
  - Incubation – increasing MICs with longer incubation
  - Atmosphere – affects the activity of some drugs
  - pH – some drugs are more active at high pH, others at low

The **median** of the MIC (or zone diameter) distribution:

- The inherent susceptibility of the species to the drug
- Anything systematically influencing the activity of the drug
  - Medium, inoculum, pH, cations, incubation atmosphere and time,

The **width** of the MIC (or zone diameter) distribution:

- Inherent variation in susceptibility to the drug
- Biological variation in other traits that influence the MIC
  - any biological characteristic such as generation time, nutrient dependency, atmosphere dependency etc.
- Exogenous variation randomly influencing the activity of the drug
  - pH, cations, incubation atmosphere and time, etc.
- Variation in reading (between days, between readers, between systems)
- The stability of the molecule
  -
Kahlmeter - ECOFFs, ECOFFs, ECOFFs

S.aureus - clinical isolates vs S.aureus ATCC 29213
125 clinical isolates vs. 125 determinations of one type strain

How much of the variation in the wild type is due to general variation and how much pertains to variation in sensitivity to the drug?

EUCAST workshop, ECCMID 2013

MIC distributions in zone diameter histograms
Linezolid

No. of observations

S. aureus  E. faecalis  S. pneumoniae

Inhibition zone (mm)

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

Sjölund et al, CMI, 2009

MIC (mg/L)

Sahand et al, OAFL, 2009
Methods for defining the wild type distribution (determining the ECOFF)

- The “eyeball” method (Kahlmeter)
- The 95% rule (Pfaller)
- The Normalised Resistance Interpretation (Kronvall)
- The iterative statistical method (Turnidge)
- Multimodal analysis (Meletiadis)
The Normalized Resistance Interpretation

• Uses an adaptation of a method originally devised for “ECOFFs” for zone diameter distributions  
  — Kronvall et al., Clin Micro Infect 2003

From John Turnidge, CLSI Workshop, Tampa, January 2013

Iterative Statistical Method

ORIGINAL ARTICLE

Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values

From John Turnidge, CLSI Workshop, Tampa, January 2013

Iterative Statistical Method - COFinder

From John Turnidge, CLSI Workshop, Tampa, January 2013
"A biological approach"

- Associated resistance – an organism resistant to one agent is very likely to carry resistance to other agents.
- By comparing the MIC distributions of all isolates and isolates wild type to all other agents.
The use of ECOFFs (1)

1. As a tool in the determination of clinical breakpoints
   - To avoid dividing wild type MIC distributions of target organisms
   - As a surrogate clinical breakpoint when clinical data pertain only to wild type organisms (and when PK/PD data are incomplete).

2. For sensitive detection of (screening for) resistance
   - oxacillin to detect all penicillin-R in S. pneumoniae
   - cefoxitin to detect methicillin resistance in S. aureus
   - benzylpenicillin to detect all beta-lactam resistance in H.influenzae
   - pefloxacin to detect quinolone resistance in Salmonella spp
   - meropenem to screen for KPC in Enterobacteriaceae

EUCAST workshop, ECCMID 2013

The use of ECOFFs (2)

3. For surveillance of antimicrobial resistance when clinical breakpoints...
   - have not been determined
   - are not sensitive enough
   - change over time
   - differ between systems (CLSI, FDA, EUCAST etc)
   - differ between humans, cows, pigs, birds, fish and camels.

4. To exclude resistance
   - To exclude methicillin resistance, carbapenem resistance etc
   - food safety – in the development of functional foods

5. For clinical susceptibility reports?

EUCAST workshop, ECCMID 2013

Is it possible to report the WT/NWT status of an isolate as supplementary information to S, I and R

- *E. faecalis* with MIC 16 mg/L to gentamicin would be reported: R<sup>WT</sup>
- *E. coli* with MIC 0.5 mg/L to ciprofloxacin would be reported: S<sup>NWT</sup>
- *E. faecalis* with MIC 1.0 mg/L to daptomycin would be reported: IE<sup>WT</sup>
- *K. pneumoniae* with MIC 1.0 mg/L (and a KPC) would be reported: S<sup>NWT</sup>

EUCAST workshop, ECCMID 2013
The background

- Guidance on methods of detection and characterization of resistance mechanisms are required to tie in with
  - The EUCAST MIC breakpoints
  - The EUCAST disk diffusion method
  - EUCAST Expert Rules
  - The ECDC requirements for update of the EARS-Net manual

The remit

- To develop practical guidelines for detection of specific antimicrobial resistance mechanisms of clinical and/or epidemiological importance.
- The guidance will include:
  - Definition of the mechanisms.
  - Explanation of the clinical and/or public health need for detection of the mechanisms.
  - An outline description of recommended methods of detection.
  - References to detailed descriptions of the methods.
- Draft guidelines that have been subject to wide consultation via established EUCAST procedures and ECDC focal points
Mechanisms and bacteria

- Methicillin-resistant *S. aureus*
- Vancomycin low-level resistance in *S. aureus* (VISA/heteroVISA)
- Vancomycin-resistant enterococci
- Penicillin non-susceptible *S. pneumoniae*
- Extended-spectrum β-lactamase producing Enterobacteriaceae
- Acquired AmpC-producing Enterobacteriaceae
- Acquired carbapenemases in Enterobacteriaceae

Members of the SC

- Christian G. Giske (Chair; Sweden, EUCAST and EARS-Net)
- Luis Martinez-Martinez (Spain)
- Rafael Canton (Spain and EUCAST)
- Stefania Stefani (Italy)
- Robert Skov (Denmark)
- Yonni Glupczynski (Belgium)
- Patrice Nordmann (France)
- Mandy Wootton (UK)
- Vivi Miriagou (Greece)
- Gunnar Skov Simonsen (Norway and EARS-Net)
- Helena Zemlickova (Czech republic and EARS-Net)
- James Cohen-Stuart (Netherlands)
- Marek Gniadkowski (Poland)
What have we achieved?

- First consultation was very fruitful – many useful comments
- Document is out on the second consultation
- Following the second consultation we expect to be able to produce a final version that can be approved by EUCAST SC in July 2013
- Highly probable that (annual?) revisions will be needed
- Publication in CMI is planned (autumn 2013)

How has the work been carried out?

- Just one initial meeting at ECCMID
- Mostly e-mail contacts with circulation of drafts
- Systematic literature searches in addition to authors’ personal experience
- Methods evaluated in multi-centre studies preferred
- Gram-positive and Gram-negative subgroups, which have both reported to the chairman

The guidelines
2. CARBAPENEMASES IN ENTEROBACTERIACEAE

Importance of detection of resistance mechanism

<table>
<thead>
<tr>
<th>Required for:</th>
<th>Infection control</th>
<th>Public Health</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARBAPENEMASE testing</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

2.1 Definition
Carbapenemases are β-lactamases hydrolysing penicillins, in most cases cephalosporins, and to varying degrees carbapenems and monobactams (the latter are not hydrolyzed by most β-lactamases).

When should screening be carried out?

<table>
<thead>
<tr>
<th>Carbapenem</th>
<th>D(10 mm)</th>
<th>S(15 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>≤12</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>10.5</td>
<td>&gt;10.5</td>
</tr>
</tbody>
</table>

When screening should be performed is determined by local epidemiology and institutional guidelines. A high number of patients with carbapenem resistance may indicate that screening for carbapenemases should be performed. Screening for carbapenemases is strongly recommended in patients with respiratory infection, abdominal infections, or after hospitalization in an intensive care unit.

Phenotypic confirmation methods

<table>
<thead>
<tr>
<th>Carbapenem</th>
<th>MIC (mg/L)</th>
<th>Sensitivity to carbapenem</th>
<th>Sensitivity to β-lactamase inhibitor</th>
<th>Sensitivity to β-lactamase inhibitor + MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>≤1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤0.5</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤0.5</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1 Sensitivity is also relevant in cases where a synergy is observed, in order to differentiate between ESKA, dual carbapenemase and single carbapenemase resistance.
2 When testing for synergy, it is important to use a high level of clavulanic acid (32 μg/mL) and penicillin (1 μg/mL), as these may be the most effective inhibitors of these interactions.
Giske - Detection of resistance mechanisms. When, how and why?

**EUCAST - Detection of resistance mechanisms**

**ESBL SCREENING:**
- Cefotaxime I/R and/or Ceftazidime I/R using EUCAST breakpoints

**ESBL CONFIRMATION 1:**
- If cefoxitin MIC > 8 mg/L, perform cefepime +/- clavulanic acid confirmation test
- Genotypic or phenotypic confirmation of acquired AmpC is recommended in group 1 Enterobacteriaceae isolates with negative ESBL confirmation test.
- **Group 1:**
- **Group 2:**
  - Enterobacteriaceae with inducible chromosomal AmpC: Enterobacter spp., Citrobacter freundii, Morganella morganii, Providencia spp, Serratia spp., Hafnia alvei.

**ESBL CONFIRMATION:**
- With cefepime +/- clavulanic acid
- Species dependent ESBL confirmation
  - Negative: No ESBL
  - Off range
  - Positive: ESBL
  - Negative: no ESBL
  - Off range

**When should ESBL-testing be done?**

<table>
<thead>
<tr>
<th>Method</th>
<th>Antibiotics</th>
<th>Conduct ESBL confirmation if</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil dilution</td>
<td>cefoxitin (MC: 8 mg/L)</td>
<td>Cefotaxime (MC: 2 mg/L)</td>
</tr>
<tr>
<td>Agar dilution</td>
<td>cefotaxime (MC: 1 mg/L)</td>
<td>Cefotaxime (MC: 2 mg/L)</td>
</tr>
<tr>
<td>Disk diffusion</td>
<td>cefotaxime (10 μg)</td>
<td>Disks with cefotaxime (10 μg)</td>
</tr>
<tr>
<td>Automated systems</td>
<td>cefotaxime (MC: 1 mg/L)</td>
<td>Cefotaxime (MC: 2 mg/L)</td>
</tr>
</tbody>
</table>
Giske - Detection of resistance mechanisms. When, how and why?

**Method**
- **Antibiotic Disk/tablet load**
- **Confirmation is positive if**
  - **Etest ESBL**
    - Cefotaxime +/- clavulanic acid
    - MIC ratio ≥ 8 or deformation ellipse / phantom zone present
  - **Ceftazidime +/- clavulanic acid**
    - MIC ratio ≥ 8 or deformation ellipse / phantom zone present
  - **Combination disk diffusion test (CDT)**
    - Cefotaxime +/- clavulanic acid
    - Cefotaxime 30 ug +/− clavulanic acid 10 ug ≥ 5 mm increase in inhibition zone
  - **Ceftazidime +/- clavulanic acid**
    - Ceftazidime 30 ug +/− clavulanic acid 10 ug ≥ 5 mm increase in inhibition zone
  - **Broth microdilution**
    - Cefotaxime +/- clavulanic acid
    - MIC ratio ≥ 8
  - **Ceftazidime +/- clavulanic acid**
    - MIC ratio ≥ 8
  - **Cefepime +/- clavulanic acid**
    - MIC ratio ≥ 8
  - **Double disk synergy test (DDST)**
    - Cefotaxime, ceftazidime and cefepime
    - Expansion of indicator cephalosporin inhibition zone towards amoxicillin-clavulanic acid disc

**Group II Enterobacteriaceae**

- **Screening is positive if**
  - **Etest ESBL**
    - Cefepime +/- clavulanic acid
    - MIC ratio ≥ 8 or deformation ellipse / phantom zone present
  - **Combination disk diffusion test**
    - Cefepime +/- clavulanic acid
    - Cefepime 30 ug ≥ 5 mm increase in inhibition zone
  - **Clavulanic acid 10 ug**
  - **Broth microdilution**
    - Cefepime +/- clavulanic acid
    - MIC ratio ≥ 8
  - **Double disk synergy test (DDST)**
    - Cefotaxime, ceftazidime, cefepime
    - Expansion of indicator cephalosporin inhibition zone towards amoxicillin-clavulanic acid disc

---

5. DETECTION OF METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS (MRSAs)

**Importance of detection of resistance**
- Required for antimicrobial susceptibility categorisation: Yes
- Infection control: Yes
- Public health: Yes

**Definitions**
- S. aureus isolates with an auxiliary protein binding site (DSBP) or the recently discovered (MRPs) to which [plasmid agents, except for the novel class of cephalosporins having anti-MRSA activity, have little or no affinity.**
Giske - Detection of resistance mechanisms. When, how and why?

Table 1. Interpretation of disc diffusion and e-test results

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>No resistance detected</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Intermediate resistance</td>
</tr>
<tr>
<td>Resistant</td>
<td>Resistance detected</td>
</tr>
</tbody>
</table>

6. NON-INSENSITIVITY TO GLYCOPROTEINS IN STAPHYLOCOCCI: JUNIUS

6.1 Identification
- Staphylococcus resistant to vancomycin (MIC > 8 mg/L).
- Staphylococcus aureus with intermediate resistance to vancomycin (MIC 4-8 mg/L).
- Staphylococcus xylosus with intermediate resistance to vancomycin (MIC 4-8 mg/L).
- Staphylococcus epidermidis with intermediate resistance to vancomycin (MIC 4-8 mg/L).

8.4 Recommended methods for detection

Disk-diffusion can **NOT** be used to test for either HGBA or NOBS.

8.4.1 EARS determination
- EARS using EUCAST methodology is the gold standard and is preferred to HMB.
- EUCAST methodology may also be determined by gradient strips, agar diffusion or automated systems.
- It should be noted that the results obtained by HMB are usually higher than the results obtained by EUCAST.

8.4.2 Specific tests for NOBS
- NOBS is detected by measuring the MIC, but this is not the case for HGBA. Detection of NOBS is more difficult and depends on the level of background knowledge and experience.
Giske - Detection of resistance mechanisms. When, how and why?

7. DETECTION OF VANCOMYCIN RESISTANCE IN ENTEROCOCCUS FASCIATUS AND ENTEROCOCCUS FAECALIS

<table>
<thead>
<tr>
<th>Importance of detection of resistance</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required for antimicrobial susceptibility categorisation</td>
<td>Yes</td>
</tr>
<tr>
<td>Infectious control public health</td>
<td>No</td>
</tr>
<tr>
<td>Public health</td>
<td>Yes</td>
</tr>
</tbody>
</table>

7.1 Definition

Vancomycin resistance can be detected by E-test, broth microdilution, disk diffusion and the E-test agar plate method. For all these methods it is essential that plates are incubated for 48-72h in order to capture inducible resistance.

At these methods usually detect vancomycin resistance. Detection of vancomycin resistance is more challenging. MIC determination using agar or broth dilution works with high accuracy but is seldom used in routine laboratories. Reports show that detection of vancomycin resistance in patients for autotransplantation methods (Garam, liver, kidney) one diffusion using a top vancomycin disk perform well provided the guidelines for reading as specified by EUCAST are followed meticulously (data from EUCAST Reference Laboratory, Wageningen).

8. DETECTION OF PENICILLIN NON-SUSCEPTIBILITY IN STREPTOCOCCUS PNEUMONIAE

<table>
<thead>
<tr>
<th>Importance of detection of resistance</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Required for antimicrobial susceptibility categorisation</td>
<td>Yes</td>
</tr>
<tr>
<td>Infectious control public health</td>
<td>No</td>
</tr>
<tr>
<td>Public health</td>
<td>Yes</td>
</tr>
</tbody>
</table>

8.1 Definition

If penicillin isolates with reduced susceptibility (non-vulnare MDR) to penicillin due to the presence of modified penicillin binding proteins (PBP) with lower affinity to β-lactam.
Pan-European guidelines are soon to be available

Scrutiny and constructive feedback from national methodology committees is pivotal to ensure that the guidelines are improved over time

My prediction: there will be a need for this work also beyond July 2013

European standardization may be helpful for EARS-Net, but hopefully even more so for laboratories, patients and infection control
Report as tested

- Strictly apply the recommended breakpoints for MIC or inhibition zone values for clinical categorization as susceptible (S), intermediate (I) or resistant (R)
  - to achieve the goal of having breakpoints
  - to avoid implementing expert rules
  - to avoid delay in reporting

Although breakpoints are different, report “as tested” is currently recommended by both CLSI and EUCAST committees

**Breakpoint definition (ISO 20776-1:2006)**

... beyond CLSI and EUCAST

Values of parameters, such as MICs, on the basis of which bacteria can be assigned to the clinical categories “susceptible”, “intermediate” and “resistant”

**Susceptible** bacterial strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic success

**Intermediate** bacterial strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with uncertain therapeutic effect

**Resistant** bacterial strain inhibited *in vitro* by a concentration of an antimicrobial agent that is associated with a high likelihood of therapeutic failure
Canton - Report as tested

Microbiological and clinical breakpoints

- The aim of clinical breakpoints is to use MIC values …
  - to separate strains where there is a high likelihood of treatment success from those where treatment is more likely to fail
  - to adequately treat patients but not to detect resistance mechanisms from a microbiological point of view

- They are ultimately derived from human clinical studies comparing outcomes with the MICs for the infecting pathogen

- If clinical breakpoints are well established no actions (expert rules) are needed beyond MIC interpretation (interpretive reading)

... but this has not been the case in the past!

Clinical breakpoints: the philosophy

Interpretive reading of AST results

- During more than twenty years interpretive reading of the antibiogram has been used to:
  - infer resistance mechanisms behind resistant phenotypes
  - identify resistant organisms for infection control purposes
  - apply expert rules* and modify (when needed!) previous clinical categorization

This approach was partially needed due to inadequate breakpoints!

*Action to be taken (normally S or I to R), based on current clinical or microbiological evidence, in response to specific AST results
Interpretive reading of AST results

- Interpretative reading: *the classical example*

![ESBL positive isolate](image)

- Expert rule

resistant to all cephalosporins and aztreonam (irrespective of MICs)

---

Report as tested

Q1

---

Report as tested

Q2
Canton - Report as tested

### MIC testing versus detection of resistance

- Both CLSI and EUCAST decided in separate processes (2009-10) to modify breakpoints for extended spectrum cephalosporins, based on:
  - harmonization process (only in EUCAST)
  - MIC distribution of isolates with and without ESBLs, pAmpC, …
  - animal infection models with isolates with and without ESBLs
  - PK/PD calculations (Monte Carlo simulation, …)
  - clinical results available at the time of setting breakpoints

MacGowan A. Clin Microbiol Infect 2008; 14 (Suppl 1):166-8

NO ESBL CONFIRMATION IS NEEDED UNLESS FOR EPIDEMIOLOGICAL OR INFECTION CONTROL PURPOSES

### 3rd/4th gen. cephalosporin breakpoints in Enterobacteriaceae

<table>
<thead>
<tr>
<th>Cephalosporins</th>
<th>CLSI (2010-13)</th>
<th>EUCAST (2009-13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≤1</td>
<td>≥2</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤1</td>
<td>≥4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≤4</td>
<td>≥16</td>
</tr>
<tr>
<td>Cefepime</td>
<td>≤8</td>
<td>≥32</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≤4</td>
<td>≥16</td>
</tr>
</tbody>
</table>

*2009

This approach was extended to breakpoints for carbapenems in Enterobacteriaceae

### 3rd/4th gen. cephalosporin breakpoints in Enterobacteriaceae

- CLSI and EUCAST “new” breakpoints were supported by PK/PD data, animal models and clinical outcome data

Enterobacteriaceae in a murine thigh infection model: Cephalosporin % T>MIC and microbiological efficacy

Monte-Carlo simulations and target attainment rate (TAR) for intravenous ceftriaxone 2 g every 24 h

Andes & Craig. CMI 2005; 11(Suppl 6):10-7
MacGowan. CMI 2008; 14(Suppl 1):166-8
Canton - Report as tested

3rd/4th gen. cephalosporin breakpoints in Enterobacteriaceae

- Probability of target attainment (PTA) for ceftazidime
  - 2 log drop in viable Gram-negatives requires 50% FT-MIC
  - PTA achieved for MIC of criteria
    - 1 g x 3 IV  4 mg/L  S
    - 2 g x 3 IV  8 mg/L  R

- EUCAST decreased ceftazidime and cefepime breakpoints due to evidence on clinical and MIC correlations:
  - ≤ 1 mg/L: no difference ESBL and non-ESBL producers
  - 2-4 mg/L: variable successful outcomes
  - >4 mg/L: poor outcomes

Paterson et al. JCM 2001; 39:2206-12; Andes & Craig. CMI 2005; 11 (Suppl. 6):10-7
Bin et al. DMID 2006; 56:351-7; Bhat et al. AAC 2007; 51:4365-6

3rd/4th gen. cephalosporin breakpoints in Enterobacteriaceae

- Clinical data for ESBL producers indicates that outcome success decreases when 3rd gen cephalosporins are ≥2 mg/L

Clinical outcome in patients with ESBL-producing Klebsiella spp. or E. coli bacteraemia and treated with 3rd gen. cephalosporin monotherapy

Paterson et al. JCM 2001; 39:2206-12
Andes & Craig. CMI 2005; 11 (Suppl. 6):10-7

What has been the impact of “report as tested”?

- “Theoretical” calculations (mainly on ESBLs) calculated microbiological impact on % of S-R isolates using CLSI or EUCAST breakpoints
  - Howser et al. AAC 2010; 54:3043-6; Nolte et al. AAC 2010; 54:3031-4

- Critical voices alerting on negative consequence for no further detection and reporting of ESBLs and carbapenemases
  - Livermore et al. JAC 2012; 67:1569-77; Nordmann, Poirel. JAC 2013; 487-9

- Analysis and meta-analysis of different impact on mortality for ESBL and carbapenemase producing organisms
  - Bonten et al. JAC 2012; 67:1311-20; Falagas et al. AAC 2012; 42:14-22

- New publications on clinical outcomes:
  - carbapenemase producing organisms treated with carbapenemases
    - Quereshi et al. AAC 2011; 56: 2108-13; Tzouvelekis et al.CMR 2012; 25: 682-707
    - Tumbarello et al. CID 2012; 55:943-50
Canton - Report as tested

**What has been the impact of “report as tested”?**

- Theoretical” calculations (mainly on ESBLs) calculated microbiological impact on % of S-R isolates using CLSI and EUCAST breakpoints

  - Howser et al. AAC 2010; 54:3043-6; Hoban et al. AAC 2010; 54:3031-4

  - major impact when using CLSI rather than of EUCAST
  - greater impact for ceftazidime and cefepime than for cefotaxime
  - geographic dependent impact (different ESBL epidemiology)
  - origin (hospital or community-onset) dependent impact

---

**What has been the impact of these “new” breakpoints?**

- % of ESBL-E. coli isolates susceptible to 3rd / 4th gen. ceph. when using CLSI and EUCAST breakpoints in different studies

- Impact of CLSI & EUCAST breakpoints in ESBL-E. coli blood isolates

---

**What has been the impact of “report as tested”?**

- Impact of CLSI & EUCAST breakpoints in ESBL-E. coli blood isolates

---
What has been the impact of “report as tested”?

- Ceftazidime susceptibility of prevalent CTX-M producing *E. coli*

<table>
<thead>
<tr>
<th>% of CAZ-S isolates</th>
<th>CLSI</th>
<th>EUCAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-M-14</td>
<td>93</td>
<td>74</td>
</tr>
<tr>
<td>CTX-M-15</td>
<td>11</td>
<td>2</td>
</tr>
</tbody>
</table>

Critical voices …

1. Similar number of clinical cases on record where cephalosporins and carbapenems have proved effective and ineffective against infections due to low-MIC ESBL and carbapenemase producers, respectively.

2. Routine susceptibility testing is less precise than in research: ESBL and carbapenemase producers with MICs of 1–8 mg/L will oscillate between susceptibility categories according to who tests them and how.

3. Although breakpoint committees advocate ESBL and carbapenemase detection for epidemiological purposes, some laboratories will abandon seeking these enzymes for treatment purposes, leading to a loss of critical infection control information.

Critical voices …

1. Susceptibility to carbapenems is observed for several carbapenemase producers.

2. There is a paucity of clinical successes of carbapenem-containing regimens for treating infections due to carbapenemase producers that are susceptible to carbapenems in vitro.

Detection will be useful for treating patients and for preventing nosocomial outbreaks of carbapenemase producers (and therefore MDR isolates), whatever the carbapenem resistance level is.
Canton - Report as tested

Impact of antibiotic MIC on infection outcome in patients with susceptible Gram-negative bacteria

- A higher all-cause mortality was observed for patients infected with strains with high MICs (Risk ratio 2.03; 95% CI, 1.05-3.92).

- Differences in mortality were not statistically significant in patients infected with ESBLs (Risk ratio 1.89; 95% CI, 0.94-3.92).


Bacteraemia caused by ESBL-producing Enterobacteriaceae

- ESBL production in Enterobacteriaceae causing bacteraemia is associated with higher mortality (OR 2.35; 95% CI, 1.90-2.91), but is reduced after adjustment for inadequate empirical therapy.


Carbapenem breakpoints in Enterobacteriaceae

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤4</td>
<td>≤1 (4*)</td>
<td>≥2 (16)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤4</td>
<td>≤1 (4)</td>
<td>≥2 (16)</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤2</td>
<td>≤0.25 (2)</td>
<td>≥1 (8)</td>
</tr>
<tr>
<td>Doripenem</td>
<td>≤0.5</td>
<td>≤1 (ND)</td>
<td>≥2 (ND)</td>
</tr>
</tbody>
</table>

*2009; **E. coli y K. pneumoniae; ND: not defined

EUCAST breakpoint are higher than those of CLSI!

What is the clinical impact?
Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria

Efficacy of antimicrobial regimens used to treat infections caused by carbapenemase-producing Klebsiella pneumoniae


<table>
<thead>
<tr>
<th>Antibiotic regimen</th>
<th>No. of patients (%)</th>
<th>Outcome success (%)</th>
<th>Failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>64 (47.2)</td>
<td>75 (54.7)</td>
<td>29 (20.5)</td>
</tr>
<tr>
<td>Pipemidicin</td>
<td>6 (8.7)</td>
<td>5 (6.5)</td>
<td>5 (6.5)</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>2 (2.8)</td>
<td>2 (2.8)</td>
<td>2 (2.8)</td>
</tr>
<tr>
<td>Total</td>
<td>72 (100)</td>
<td>82 (100)</td>
<td>37 (51.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibiotic regimen</th>
<th>No. of patients (%)</th>
<th>Outcome success (%)</th>
<th>Failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination therapy</td>
<td>53 (23.2)</td>
<td>38 (73.1)</td>
<td>14 (26.9)</td>
</tr>
<tr>
<td>Two or more active drugs</td>
<td>30 (13.6)</td>
<td>26 (86.7)</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Total</td>
<td>83 (37.5)</td>
<td>64 (77.3)</td>
<td>19 (22.7)</td>
</tr>
</tbody>
</table>

Carbapenemase producing Enterobacteriaceae

Carbapenem monotherapy: 50 patients from 15 studies

Mortality in bloodstream infections and KPC-K. pneumoniae

- Higher 30-day mortality rate in patients treated with monotherapy (54.3%) that those with combination (34.1%) therapy (P=0.02)
- Significant decrease of mortality in patients treated with combination therapy including meropenem

Kaplan-Meier curves (survival)
Canton - Report as tested

**Mortality in bloodstream infections and KPC-K. pneumoniae**

- Higher 30-day mortality rate in patients treated with monotherapy (54.3%) than those with combination (34.1%) therapy ($P=0.02$)
- Significant decreased of mortality in patients treated with combination therapy including meropenem

- Kaplan-Meier curves (survival)

- Mortality (%): combination therapy


**What is the impact of carbapenem MIC values?**

**Mortality in bloodstream infections and KPC-K. pneumoniae**

- 30-day mortality rate in patients treated with combination therapy including meropenem stratified by meropenem MIC values

- MIC testing versus detection of resistance

*59*
Canton - Report as tested

**MIC testing versus detection of resistance**

Some additional issue

- Hetero-resistance, particularly in carbapenemase producers
- Different expression of ESBL and carbapenemase resistance genes
- Presence of ESBL and carbapenemase resistance genes in isolates within the wild type population (silent expression)
- Still waiting additional MIC correlations with clinical outcomes

---

**REPORT AS TESTED**

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Dr. Rafael Canton
Hospital Universitario Ramón y Cajal
Departamento de Microbiología y Virología

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Departamento de Microbiología III
Universidad Complutense, Madrid
Mouton - What are PK/PD breakpoints?

What are PK/PD breakpoints?

Johan W. Mouton MD PhD FIDSA
Professor pharmacokinetics and pharmacodynamics

LAB REPORT

- Provides Clinician/Consultant guidelines how to optimally treat a patient (Freely translated from EUCAST guideline)

Is susceptibility (MICs) related to (clinical) outcome?

If yes, which values (breakpoints) make the difference?
Mouton - What are PK/PD breakpoints?

Potency of a drug in vitro (MIC) → Exposured to the bug in vivo (PK) → Dosing Regimen

Antimicrobial Efficacy of the Drug (Microbiological Cure) → Effect on Host (Clinical Cure)

---

**Potency**

- Lowest concentration with no visible growth after 18 hour incubation

- MIC

- PK

- X-acin 500 mg

- AUC is usually linearly related to Dose

**Pharmacokinetic parameters:**

- Measures of Exposure

**Dosing Regimen**

- MIC = 2 mg/L
What are PK/PD breakpoints?

Pharmacodynamic index (AUC/MIC, Peak/MIC, T>MIC)

Probability of cure after treatment with fluconazole
Oropharyngeal Candidiasis  n=132

• Prob cure correlates with AUC/MIC
• POSITIVE correlation with EXPOSURE
• INVERSE correlation with MIC

• If AUC is known because of the standard dose e.g. 400 mg ~ 400 mg.h/L
• And an AUC/MIC of 100 is required
• It follows that the breakpoint is 400/100 = 4 mg/L

Rodriguez- Tudela et al, AAC 2007
Susceptible (S)
A micro-organism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success. A micro-organism is categorized as susceptible by applying the appropriate breakpoint in a defined phenotypic test system.

Note: This breakpoint may be altered with legitimate changes in circumstances.

Intermediate (I)
A micro-organism is defined as intermediate by a level of antimicrobial activity associated with indeterminate therapeutic effect. A micro-organism is categorized as intermediate by applying the appropriate breakpoints in a defined phenotypic test system.

Note: This breakpoint may be altered with legitimate changes in circumstances.

Resistant (R)
Bacteria are defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure. A micro-organism is categorized as resistant by applying the appropriate breakpoint in a defined phenotypic test system.

Note: This breakpoint may be altered with legitimate changes in circumstances.

WE AIM FOR:
A high likelihood of success for every one (S)
Hitting the PK/PD target

SETTING A BREAKPOINT –PK/PD
(example 1)

DETERMINE THE PK/PD TARGET  e.g. value of the PK/PD Index

ESTIMATE EXPOSURE  from the dosing regimen and PK, including population variability

CALCULATE PK/PD BREAKPOINT  from  PK/PD target = PK/PD Index
Mouton - What are PK/PD breakpoints?

**Relationship between \( fAUC/MIC \) and Effect**

121 patients with *S. pneumoniae* respiratory infection

- \( fAUC/MIC \) cut-off \( \sim 34 \)

- Relationship between \( fAUC/MIC \) ratio & microbiological response from a total 121 patients with respiratory tract infection involving *S. pneumoniae*.
- \( fAUC:MIC > 34 \) had 92.6% response rate.
- \( fAUC:MIC < 34 \) had 66.7% response rate.

Be sure that the \( fAUC/MIC \) ratio is at least appr. 34 in every patient

\[ \text{GOOD Clinical Practice} \]

**Levofloxacin 500 mg**

\[ fAUC = 30-50 \text{ mg/L} \]
Mouton - What are PK/PD breakpoints?

Clinical practice:

When starting treatment, we do not know:

- the AUC in the individual patient

Pharmacokinetics

Some people are more equal than others...

fAUC distribution levofloxacin

(monte carlo simulation)
Mouton - What are PK/PD breakpoints?

The fAUC is calculated for 10,000 patients using MCS. This results in a probability distribution of AUCs. The $fAUC/MIC$ is calculated for each MIC.

On the average, this duck is dead.
Mouton - What are PK/PD breakpoints?

The fAUC is calculated for 10,000 patients using MCS.
This results in a probability distribution of AUCs.
The fAUC/MIC is calculated for each MIC.

levofloxacin 500 mg x 1 oral

MIC mg/L

fAUC/MIC

95% percentile
99% percentile
mean

Mouton et al., 2004

The fAUC is calculated for 10,000 patients using MCS.
This results in a probability distribution of AUCs.
The fAUC/MIC is calculated for each MIC.

levofloxacin 500 mg x 1 oral

MIC mg/L

fAUC/MIC

95% percentile
99% percentile
mean

Mouton et al., 2004

The fAUC is calculated for 10,000 patients using MCS.
This results in a probability distribution of AUCs.
The fAUC/MIC is calculated for each MIC.

levofloxacin 500 mg x 1 oral

MIC mg/L

fAUC/MIC

95% percentile
99% percentile
mean

Mouton et al., 2004
Mouton - What are PK/PD breakpoints?

The fAUC is calculated for 10,000 patients using MCS. This results in a probability distribution of AUCs. The fAUC/MIC is calculated for each MIC.

levofloxacin 500 mg x 1 oral

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levofloxacin 500 mg x 1 oral

The fAUC is calculated for 10,000 patients using MCS. This results in a probability distribution of AUCs. The fAUC/MIC is calculated for each MIC.

levofloxacin 500 mg x 1 oral
Mouton - What are PK/PD breakpoints?

Levofloxacin / Streptococcus pneumoniae
Antimicrobial wild type distributions of microorganisms – reference database

High dose levofloxacin
2x 500 mg, or 750 mg
AUC 70-80

High dose levofloxacin
2x 500 mg, or 750 mg
AUC 70-80

Target
35 = 70 / 2
Mouton - What are PK/PD breakpoints?

GOOD Clinical Practice

Be sure that the fAUC/MIC ratio is at least appr. 34 in every patient

This includes patients with a high clearance

Bugs with MICs that can be expected

SETTING A BREAKPOINT –PK/PD (example 2)

DETERMINE THE PK/PD TARGET  e.g. value of the PK/PD Index

ESTIMATE EXPOSURE  from the dosing regimen and PK, including population variability

CALCULATE PK/PD BREAKPOINT  from  PK/PD target = PK/PD Index
Mouton - What are PK/PD breakpoints?

**Ceftazidime in patients with nosocomial pneumonia**
- Randomized, double-blind phase 3 clinical trial (NCT00210964):
  - Comparing the efficacy of ceftobiprole with the combination CAZ and linezolid
  - Ceftazidime 3dd 2 gr 2h infusion
  - Extensive and sparse sampling of ceftazidime

  - N=390 patients included
  - 16 without PK estimates
  - N=170 with MIC
  - 220 without Gram negatives in cultures
  - N=154 with MIC and PK-estimates

  Muller et al, JAC 2013 68:900-906

**Exposure-response Emax model**
- Individual exposures to CAZ
- Categorised (%T>MIC per 10%)
- Eradication rate per group
- 154 patients

Muller et al, JAC 2013 68:900-906

**Ceftazidime in patients with nosocomial pneumonia**
- CART analysis
  - %T>MIC breakpoint = 44.9 %
  - P< 0.0001
  - %T>MIC | Success | Failure
  - --- | --- | ---
  - >44.9 | 83 (90.2%) | 9 (9.8%)
  - <44.9 | 31 (50%) | 31 (50%)

Muller et al, JAC 2013 68:900-906
Mouton - What are PK/PD breakpoints?

**Susceptible (S)**

A micro-organism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success. A micro-organism is categorized as susceptible by applying the appropriate breakpoint in a defined phenotypic test system. Note: This breakpoint may be altered with legitimate changes in circumstances.

**Intermediate (I)**

A micro-organism is defined as intermediate by a level of antimicrobial activity associated with indeterminate therapeutic effect. A micro-organism is categorized as intermediate by applying the appropriate breakpoints in a defined phenotypic test system. Note: This breakpoint may be altered with legitimate changes in circumstances.

**Resistant (R)**

Bacteria are defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure. A micro-organism is categorized as resistant by applying the appropriate breakpoint in a defined phenotypic test system. Note: This breakpoint may be altered with legitimate changes in circumstances.

**Probability of Target Attainment - Ceftazidime**

- 100% percentile
- 95% percentile
- Mean
- MIC mg/L

BP = 4 mg/L

**EUCAST rationale document**

EUCAST: European Committee on Antimicrobial Susceptibility Testing

European Society of Clinical Microbiology and Infectious Diseases
Mouton - What are PK/PD breakpoints?

Implications for breakpoints

Susceptibility (MICs) are related to (clinical) outcome

Susceptibility (MICs) are related to (clinical) outcome?

Breakpoint values make the difference – but include PK!!!
**Conclusions**

- PK/PD breakpoints reflect the relationship between exposure and clinical outcome
- PK/PD breakpoints are dependent on dose (!), pharmacokinetic profile and pharmacodynamic target
- The pharmacodynamic target MAY differ by species (e.g. Gram- vs Gram+)
- EUCAST PK/PD breakpoints are based on clinical data if available and otherwise on animal data and other data. Rationale documents describe the background.
Phenotypic laboratory testing does not determine:

1) Likely adverse events
2) Likely drug interactions
3) Cost

In addition to phenotypic susceptibility these factors will determine patient outcomes and drug acceptability.
Pharmacodynamics and Clinical Breakpoints

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>Percentage of S. aureus strains</th>
<th>Healthy volunteers</th>
<th>Infected patients (SSTI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>0.4%</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.12</td>
<td>16.3%</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.25</td>
<td>63.6%</td>
<td>100</td>
<td>90.7</td>
</tr>
<tr>
<td>1.0</td>
<td>26.7%</td>
<td>100</td>
<td>90.3</td>
</tr>
<tr>
<td>2.0</td>
<td>-</td>
<td>43</td>
<td>39.7</td>
</tr>
<tr>
<td>4.0</td>
<td>-</td>
<td>0</td>
<td>3.4</td>
</tr>
<tr>
<td>8.0</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

EUCAST clinical breakpoint S ≤ 1mg/L

Assumptions in PK-PD approach to clinical breakpoints

- Based on serum pharmacokinetics
  - What about other infection sites? IV or oral switch if poor bioavailability
- Based on standard patients
  - (Tertically ill, obese, infected, etc)
- Based on standard drug dosing
  - (Bolus injection vs continuous infusion or prolonged infusion - B-lactams)
- MIC accurately reflects susceptibility or resistance mechanism
  - (VISA, hVISA, efflux pumps and fluoroquinolones).

Pharmacokinetic issues

**Intravenous/oral administration**

Amoxicillin 2g 8hrly IV switch to 500mg 8hrly po, bioavailability 75-92%

Target attainment rates for daptomycin 4mg/kg/day, target AUC24 total/MIC ratio ≥ 348 (static effect; maximum 12)

Table: Target attainment rate

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>Percentage of E.coli strains</th>
<th>2g 8hrly IV</th>
<th>0.5g 8hrly po</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.5</td>
<td>2.2%</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>1.6</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>31.6</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>12.7</td>
<td>91</td>
<td>1.0</td>
</tr>
<tr>
<td>≥16</td>
<td>39.7</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

EUCAST clinical breakpoints

- M.catarrhalis S ≤ 1mg/L (+clavulanic acid)
- H.influenzae S ≤ 2mg/L
- B.haemolytic streptococci S ≤ 0.5mg/L
- S.pneumoniae S ≤ 2mg/L
- Enterobacteriaceae S - or ≤ 0.5mg/L
Pharmacokinetics
Site of infection: acute pneumococcal meningitis versus non-meningitic infection

<table>
<thead>
<tr>
<th>Clinical breakpoint</th>
<th>Meningitis</th>
<th>Other infection site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>≤ 0.06</td>
<td>≤ 2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤ 0.25</td>
<td>≤ 2</td>
</tr>
</tbody>
</table>

Peterson et al, 2004; Yu et al 2003; Weinstein et al, 2009

Critically ill patients: response by MIC in VAP

<table>
<thead>
<tr>
<th>EUCAST breakpoint</th>
<th>Ceftazidime</th>
<th>Meropenem</th>
<th>Piperacillin/Tazobactam</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>≤ 8</td>
<td>≤ 2</td>
<td>≤ 16</td>
</tr>
<tr>
<td>Entero. cloacae</td>
<td>≤ 4</td>
<td>≤ 2</td>
<td>≤ 2</td>
</tr>
<tr>
<td>&gt;4.5</td>
<td>≥ 2</td>
<td>≥ 2</td>
<td>≥ 16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>Lorente et al, 2006; Lorente et al, 2007; Lorente et al, 2008.</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.5</td>
<td>11.6% (8/69) p=0.8</td>
</tr>
<tr>
<td>&gt;0.5</td>
<td>33.3% (6/18) p=0.002</td>
</tr>
<tr>
<td>&gt;0.25</td>
<td>6.3% (3/50)</td>
</tr>
</tbody>
</table>

Critical illness: Blood Stream Infection due to E. coli or K. pneumoniae producing ESBLs: ertapenem

<table>
<thead>
<tr>
<th>ertapenem MIC (mg/L)</th>
<th>Septic related mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥0.5</td>
<td>11.6% (8/69)</td>
</tr>
<tr>
<td>&gt;0.5</td>
<td>33.3% (6/18) p=0.002</td>
</tr>
</tbody>
</table>

Lee et al, 2012

EUCAST clinical breakpoint ≤0.5mg/L.
MacGowan - Are all S equal?

**Ertapenem pharmacokinetics in critically ill patients**

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers</th>
<th>Critically ill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (mg/L)</td>
<td>253</td>
<td>53</td>
</tr>
<tr>
<td>AUC(0→∞) (mg/Lh)</td>
<td>817</td>
<td>188</td>
</tr>
<tr>
<td>Vss (L)</td>
<td>9.7</td>
<td>26.8</td>
</tr>
</tbody>
</table>

Unbound fT>MIC ≤30% in 25% of patients

Brink et al, 2009

**Critical illness: Blood stream infection caused by Gram-negative organisms. Levofloxacin (mg/L)**

<table>
<thead>
<tr>
<th>Levofloxacin MIC (mg/L)</th>
<th>0.25 mg/L</th>
<th>0.5 mg/L</th>
<th>≥1 mg/L</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>12.6%</td>
<td>11.3%</td>
<td>14.3%</td>
<td>0.91</td>
</tr>
<tr>
<td>Length of stay post culture</td>
<td>7.3</td>
<td>7.9</td>
<td>16.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Length of levofloxacin therapy post culture</td>
<td>4.8</td>
<td>6.2</td>
<td>13.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Duration of infection (days)</td>
<td>1.0</td>
<td>1.2</td>
<td>2.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

De Fife et al, 2009

EUCAST clinical breakpoint ≤1 mg/L.

Cmax, Cmin, t1/2, AUC0 were all significantly different to healthy volunteers in critical care for levofloxacin

Rebuck et al, 2002

**Non-standard dosing: continuous infusion (CI)/prolonged infusion (PI)**

Doripenem 500mg IV by 4hr infusion (PI), 8hr vs imipenem 500mg 30 min infusion (II) 6hr in VAP patients.

MIC ≤4mg/L
- doripenem 97% isolates
- imipenem 91% isolates

For E.coli, Klebsiella sp, P.aeuruginosa and Enterobacter cloacae

<table>
<thead>
<tr>
<th></th>
<th>Doripenem PI</th>
<th>Imipenem II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical cure</td>
<td>73% (46/63)</td>
<td>55% (28/51)</td>
</tr>
<tr>
<td>Microbiologic cure</td>
<td>73% (46/63)</td>
<td>55% (28/51)</td>
</tr>
</tbody>
</table>

Chastre et al, 2008
Non-standard dosing: continuous infusion β-lactam therapy in VAP

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>Ceftazidime (n=56)</th>
<th>Meropenem (n=65)</th>
<th>Piperacillin/tazobactam (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.5</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>&gt;0.5</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>≤2</td>
<td>92</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>&gt;2</td>
<td>75</td>
<td>14</td>
<td>88</td>
</tr>
<tr>
<td>16</td>
<td>87</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>


MIC reflects susceptibility?

Intra-abdominal infection treated with moxifloxacin

<table>
<thead>
<tr>
<th>Anaerobe MIC (mg/L)</th>
<th>Clinical Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤2</td>
<td>91.2% (31/34)</td>
</tr>
<tr>
<td>4</td>
<td>82.4% (14/17)</td>
</tr>
<tr>
<td>8</td>
<td>83.3% (5/6)</td>
</tr>
<tr>
<td>16</td>
<td>66.7% (16/24)</td>
</tr>
<tr>
<td>32</td>
<td>83.3% (5/6)</td>
</tr>
</tbody>
</table>

Goldstein et al 2001

FDA/CLSI clinical breakpoint 5±2mg/L

Confounders: mixed infection; surgery; non-challenging indication-acute appendicitis, altered pharmacodynamics

Vancomycin MIC and S.aureus susceptibility: present status

EUCAST clinical breakpoint for S.aureus is sensitive ≤2mg/L for vancomycin

However—

“Based primarily on clinical evidence, those strains of S.aureus with vancomycin MICs values of 2mg/L, which are on the border of the wild type MIC distribution, including N0ISA phenotype are likely to have impaired clinical responses to vancomycin”

EUCAST vancomycin rationale document 2.1, 17th June, 2010
MacGowan - Are all S equal?

**MICs and outcome – new data (1)**

Systematic review and meta-analysis of vancomycin MICs and outcomes
Van Hal et al 2012, CID 54, 735

22 studies included: 2083 MRSA and 507 MSSA BSI

Conclusions:

- Vancomycin MIC significantly associated with mortality in MRSA infection (OR 1.64, p <0.01)
- E-test most common method of determining MIC
- 8 studies where E-test result stratified MIC as ≤1.0, ≥1.5 or ≥2mg/L. MIC ≥2mg/L associated with increased mortality in MRSA infection (OR 1.7, p<0.01). MIC ≤1.5mg/L not associated with increased mortality versus MIC ≤1.0mg/L

**MICs and outcome – new data (2)**

Teicoplanin
Chang et al 2012; JAC 67, 736-41

- MRSA bacteraemia, hospital based retrospective observational study (n=101)
- teicoplanin MIC>1.5mg/L associated with higher mortality (48.9% deaths vs 26%)
- EUCAST clinical breakpoint for *S.aureus* ≤2mg/L

**Predicting raised vancomycin MICs in MRSA**

Lubin et al 2011, CID 52, 997

Scoring System:

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Age &gt;50 years</td>
</tr>
<tr>
<td>2</td>
<td>Vancomycin for &gt;48hr in last week</td>
</tr>
<tr>
<td>2</td>
<td>Chronic liver disease</td>
</tr>
<tr>
<td>2</td>
<td>History of MRSA bacteraemia</td>
</tr>
<tr>
<td>1</td>
<td>Non-tunneled central line</td>
</tr>
</tbody>
</table>

Score ≥4 - negative predictive rate 91%
Positive predictive value 30%
Vancenycin MICs and MSSA outcomes

Holmes et al 2011, CID 204, 340

8 Australian hospitals 532 patients with S.aureus bacteraemia

Increasing vancomycin MIC associated with mortality in vancomycin treated patients

but also MSSA patients treated with flucloxacinilin (mortality 26.8% MIC >1.5mg/L, 12.2% <1.5mg/L by E.test)

and also:-

Han et al 2012, AAC, 56, 5164-5170

The problem with definitions/MICs

<table>
<thead>
<tr>
<th>Vancomycin status by PAP–AUC</th>
<th>Vancomycin MIC, µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.5</td>
</tr>
<tr>
<td>Susceptible (n=106)</td>
<td>11</td>
</tr>
<tr>
<td>hVISA (n=157)</td>
<td>0</td>
</tr>
<tr>
<td>VISA (n=20)</td>
<td>0</td>
</tr>
</tbody>
</table>

Potential clinical approaches – when to test Vancomycin susceptibility (MIC and/or PAP)

- Perform MIC on all MRSA from serious infection (blood, respiratory, IE, bone & joint) assess therapy on basis of result; maybe method dependent
- Perform MIC on all MRSA isolates: the clinical significance is not always obvious
- Perform occasional surveys of Vancomycin susceptibility on local MRSA isolates – see Bowker et al, P1582 this ECCMID
- Perform MIC testing on patients responding poorly to Vancomycin for MRSA infections i.e. persistently positive blood cultures after >5days
- Use other agents to vancomycin for severe infections, use combination therapy with vancomycin for severe infections...
Conclusions:

- Categorical susceptibility testing has to be evaluated in a clinical context: risk of adverse events, potential interactions, requirement for combination therapy.
- Pathogens reported as susceptible may not all respond the same way to different drugs or to the same drug because of patient factors or drug dosing factors.
- Not all “S” are equal.
Antifungal Susceptibility Testing (AFST) in the routine lab?

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Statens Serum Institute
Denmark

Disclosures:
Research grants & Speaker: Astellas, Gilead, MSD & Pfizer;
Advisory board: MSD, Povery, Pfizer. Acted as consultant for: Ahmad, Astellas, Gilead & Pfizer
Chair(wo)man for EUCAST-AFST
Advisor for CLSI-AFST

Question slides 1-2 Please

Q1 Which of the following statements is false?
1. Correct species identification predicts susceptibility and resistance
2. For Candida infections, AFST is most important in patients exposed to antifungal treatment
3. For Aspergillus infections, AFST is always important as azole resistant isolates are encountered in azole naïve patients
4. Susceptibility testing of Aspergillus should be done in a mycology reference laboratory

Motivation behind AFST

Why?
- Detect (unexpected) Resistance
  - Acquired - not predicted by the "name"
  - Intrinsic - isolates difficult to identify

When?
- Candida particularly AF exposed patients
- Aspergillus even azole naïve patients

How?
- Reference methods
- Commercial methods
Arendrup - Antifungal susceptibility testing in the routine lab?

Reference methods

- **CLSI**
  - M27-A3 Yeast broth dilution 120 $
  - M27-S4 QC and BPs 35 $
  - M38-A2 Mould broth dilution 120 $
  - M44-A2 Yeast disk diffusion 200 $
  - M44-S3 QC and BPs 35 $
  - M51-A Mould disk diffusion 170 $
  - M51-S1 QC and ECVs 200 $

- **EUCAST**
  - Methods EDef 7.2 (yeast) and EDef 9.1 (mould)
  - Rationale documents (breakpoints)
  - Candida: amphotericin; anidul-, micafungin; flu-, vori- and posaconazole
  - Aspergillus: amphotericin, itra-, posa- and voriconazole

Technical notes in Clin Microbiol Infect

Free


- Flat bottom plates
- RPMI with 2% glucose & MOPS
- 0.5-2.5 x 10⁵ cells/mL

<table>
<thead>
<tr>
<th>Drug dilutions</th>
<th>OD value</th>
<th>Concentration mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>N</td>
<td>PSC</td>
</tr>
<tr>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Incubation at 35 ± 2°C

24h and OD↑ by ≥ 0.2

50% inhibition, Spec. reading

AMB & Azole breakpoints for Candida spp

<table>
<thead>
<tr>
<th>Breakpoints (BPs)</th>
<th>CLSI</th>
<th>CLSI</th>
<th>Revised BPs</th>
<th>EUCAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMB</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1 - x</td>
<td></td>
</tr>
<tr>
<td>Fluco</td>
<td>≤8; &gt;32</td>
<td>&lt;2; &gt;4</td>
<td>≤0.125; &gt;0.5</td>
<td>≤0.125; &gt;0.5</td>
</tr>
<tr>
<td>SDD ≤32; R &gt;32</td>
<td>(alb, para, trop)</td>
<td>(glab)</td>
<td>(alb, para, trop)</td>
<td>(glab)</td>
</tr>
<tr>
<td>≥0.002; R &gt;32</td>
<td>(krus poor target)</td>
<td>(krus poor target)</td>
<td>(krus poor target)</td>
<td>(krus poor target)</td>
</tr>
<tr>
<td>Itra</td>
<td>≤0.125; &gt;0.5</td>
<td>≤0.125; &lt;0.5</td>
<td>≤0.06; &gt;0.06</td>
<td>(alb, para, trop)</td>
</tr>
<tr>
<td></td>
<td>(glab/krus IE)</td>
<td>(glab/krus IE)</td>
<td>(glab/krus IE)</td>
<td>(glab/krus IE)</td>
</tr>
<tr>
<td>Posa</td>
<td>≤1; &gt;2</td>
<td>≤0.125; &gt;0.5(alb, para, trop)</td>
<td>≤0.125; &gt;0.125 (alb, para, trop)</td>
<td>≤0.125; &gt;0.125 (alb, para, trop)</td>
</tr>
<tr>
<td></td>
<td>&gt;0.5; x1</td>
<td>(krus)</td>
<td>(glab)</td>
<td>(glab)</td>
</tr>
</tbody>
</table>

Breakpoints (BPs): S: ≤X; R: >Y

Revised BPs

www.eucast.org; Pfaller Drug Resist Updat. 2010 & 2011; www.clsi.org
Arendrup - Antifungal susceptibility testing in the routine lab?
Arendrup - Antifungal susceptibility testing in the routine lab?

### Commercial testing methods

- **Test performance**
  - Eg. variability in zone interpretation

- **Interpretation of endpoints**
  - Caveats adopting CLSI /EUCAST BPs
  - Echinocandins as an example
    - Etest
    - Vitek

### C. albicans. Etest.

- Amphotericin B
- Fluconazole
Arendrup - Antifungal susceptibility testing in the routine lab?

C. albicans. Etest.

Amphotericin B Fluconazole

Adopting BPs: CLSI & Echinocandin Etest

- Are Test MIC_{50,90} for each species = reference MIC?

CLSI ANF CLSI-CSF CLSI-MFG

Etest Etest Etest

Echinocandin Etest MICs & CLSI BP

C. albicans

80
43
31
5
1

C. glabrata

81
8
67
6

C. dublinensis

14
2
11
1

C. tropicalis

8
2
5
1

C. krusei

5
5
5
1

C. parapsilosis

36
11
4
13

C. guillermondii

2
11

C. lusitaniae

8
7
6
2

C. albicans*

80
36
38
6

C. glabrata

81
4
74
3

C. dublinensis

14
6
7
1

C. tropicalis

8
2
5
1

C. krusei

5
3
2

C. parapsilosis

36
28
24
2

C. guillermondii

2
2

C. lusitaniae

8
1
6
1

Micafungin Caspofungin Anidulafungin

Axner-Elings JCM 2011
Arendrup - Antifungal susceptibility testing in the routine lab?
Arendrup - Antifungal susceptibility testing in the routine lab?

**Etest: Caspofungin & CLSI BP**

- **C. albicans**
- **C. glabrata**
- **C. tropicalis**
- **C. krusei**
- **C. parapsilosis**

**FKS mutants:**
- S: 7% (2/29)
- I: 7% (2/29)

**WT as I/R:** 13% (6/47)

**MIC (μg/ml)**

0.002
0.004
0.008
0.015
0.032
0.064
0.125
0.25
0.5
1
2
4
8
16
>32

**No. of isolates**

0
1
2
3
4
5
6
7
8
9
10

---

**Etest: Caspofungin and CLSI BP**

- **C. albicans**
- **C. glabrata**
- **C. krusei**
- **C. tropicalis**

**Etest**

**CLSI**

**Breakpoint**

<table>
<thead>
<tr>
<th>Card conc.</th>
<th>MIC range reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>1, 4, 16, 32</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>1, 4, 8, 16</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>1, 4, 8, 64</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.5, 1, 4, 8</td>
</tr>
<tr>
<td><strong>NEW Caspofungin</strong></td>
<td>1, 4, 8</td>
</tr>
</tbody>
</table>

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**Caspofungin and Vitek**

**Vitek 2**

<table>
<thead>
<tr>
<th>Antifungal compound</th>
<th>MIC range reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>0.025 - 16</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0.001 - 16</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0.001 - 16</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.012 - 16</td>
</tr>
<tr>
<td><strong>NEW Caspofungin</strong></td>
<td>0.025 - 4</td>
</tr>
</tbody>
</table>

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**Card conc.**

<table>
<thead>
<tr>
<th>Breakpoint</th>
<th>EUCAST</th>
<th>CLSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>0.25/0.125</td>
<td>0.25/0.125</td>
<td></td>
</tr>
</tbody>
</table>
Arendrup - Antifungal susceptibility testing in the routine lab?

**Take home messages Commercial Tests**

- You have to check for each drug-bug combination
- BPs can only be adopted if your MICs mirror those for the ref. method!
- Recommendations for echinocandins
  - Echinocandins: Anidulafungin Etest with EUCAST BPs
  - VITEK2 problematic: MIC range doesn’t cover the BP for C. glabrata

**UK Clin Mycology Network**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Microbial lab</th>
<th>Specialised Microbial lab</th>
<th>Regional mycology lab</th>
<th>Mycology reference lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary fungal culture</td>
<td>C. albicans = non-albicans</td>
<td>Cryptococcus 3D</td>
<td>Molecular techniques</td>
<td>3 AND: ID all fungi Typing techniques National standards and training Surveillance Strain collection</td>
</tr>
<tr>
<td>Microscopy fungal elements &amp; PAP</td>
<td>3 AND: Fungal serology ID unusual fungi AFST TOAST Training programme</td>
<td>Molecular techniques</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Health care scientists: dedicated mycol

*This level of service should be available at all teaching hospitals and specialist cancer, haematology, transplant & HIV centres
Arendrup - Antifungal susceptibility testing in the routine lab?

Thank you for your attention