Redefining susceptibility categories

and introducing the "area of technical uncertainty."

Gunnar Kahlmeter

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Clinical microbiology

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In 2019 EUCAST decided ..... 

• To change the definitions of S, I and R.
• To retain the letters S, I och R.
• To emphasize the relationship between the concentration of the antimicrobial agent at the site of the infection AND the breakpoints for categorisation (S, I and R).
• To task clinical laboratories with the responsibility for uncertain laboratory results, irrespective of origin.
All breakpoints are ”exposure dependant”.

- Unless the microorganism is sufficiently exposed to the antimicrobial at the site of infection, there will be no inhibitory or killing effect.

- The degree of exposure is determined by the agent and its pharmacokinetics in the patient, the individual dose, the frequency of dosing and the mode of administration.

- For some agents there is only one dose and one mode of administration – for others there are many options.
Definitions of S and R were straightforward:

S = a micro-organism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success.

I = a microorganism is defined as intermediate by a level of antimicrobial agent activity associated with uncertain therapeutic effect. It implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used; it also indicates a buffer zone that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations.

R = a micro-organism is defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure.
• Uncertain therapeutic effect – responsibility of EMA, EUCAST and the company
  – The breakpoint committee is responsible for breakpoints and guidance.
  – The validity of indications, breakpoints and methods.

• Uncertain result – responsibility of the laboratory
  – The laboratory is responsible for AST results.
  – Methodological and/or interpretative uncertainty (failing method)

• Concentration at the site of infection – responsibility of the clinician
  – Dosing/administration: dose, frequency, mode (oral, iv, infusion).
It was impossible to know which of 3 – 4 meanings were valid.

- So INTERMEDIATE was avoided!
- We lost respect for ”INTERMEDIATE”
- We often lumped it with ”R”:
  - Some converted I to R in the report
  - Others lumped ”I” and ”R” together as ”Non-susceptible” in surveillance.
VME (false susceptible result)
ME (false resistant result)

The wider the I-category, the less likely VMEs and MEs occur. However, a wide I-category creates results with uncertain interpretation!
SIR – previous definitions

**Susceptible**

**Intermediate**
Uncertain effect.
Buffer zone for technical variation.
For a high dose,
Where concentrated for pharmacokinetic reasons.

**Resistant**
New definitions of S, I and R were needed...

**S - Susceptible, standard dosing regimen:** A microorganism is categorised as *Susceptible, standard dosing regimen*, when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.

**I – Susceptible, increased exposure:** A microorganism is categorised as *Susceptible, Increased exposure* when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.

**R - Resistant:** A microorganism is categorised as *Resistant* when there is a high likelihood of therapeutic failure even when there is increased exposure.

* Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

Gunnar Kahlmeter, EUCAST 2019
SIR – new definitions 2019

Susceptible

Resistant

Normal exposure

Increased exposure
SIR – new definitions 2019

Susceptible

Resistant
Clinical breakpoints and dosing of antibiotics
08 January 2019

Clinical breakpoints for bacteria

- Clinical breakpoints - bacteria (v 9.0) - pdf for printing (1 Jan, 2019)
- Clinical breakpoints - bacteria (v 9.0) - excel file for screen (1 Jan, 2019). For opening the Excel file, use only the original Microsoft Excel programme.

Error: in the *S. pneumoniae* tab, "oxacillin 1 unit" shall of course read "oxacillin 1 microgram".

- Clinical breakpoints - fungi (Candida and Aspergillus)
- Dosing of antibacterial agents (last tab of breakpoint table as a separate document) (1 Jan, 2019)
- What to do when there are no clinical breakpoints! Guidance from EUCAST (last edited 2016)

In the 2019 breakpoint table we highlight the following new/changed items:

- New breakpoints for meropenem-vaborbactam and eravacycline.
- Several updates due to taxonomy changes.
- Information added on EUCAST new definitions of the S, I and R categories.
- Several breakpoint changes due to the new definition of the I category. See also the Guidance on tigecycline dosing and administration.
- Trimethoprim breakpoints were removed for Enterococcus. The clinical efficacy is not predictable by AST. Using the ECOFF (now listed in a note in the table), it is however possible to predict the presence and absence of resistance mechanisms.
- Areas of Technical Uncertainties (ATUs) and information on how to handle technical uncertainty in antimicrobial susceptibility testing added.
- Flow charts for screening for beta-lactam resistance in *S. pneumoniae* and *H. influenzae* updated.
- Several updates in the dosing tab.
<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Standard dose</th>
<th>High dose</th>
<th>Special situations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbapenems</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doripenem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erlapenem</td>
<td>1 g x 1 iv over 30 minutes</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5 g x 4 iv over 30 minutes</td>
<td>1 g x 4 iv over 30 minutes</td>
<td>Pseudomonas spp.: High dose only Acinetobacter spp.: High dose only</td>
</tr>
<tr>
<td>Meropenem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Monobactams</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fluoroquinolones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.5 g x 2 oral or 0.4 g x 2 iv</td>
<td>0.75 g x 2 oral or 0.4 g x 3 iv</td>
<td>Pseudomonas spp.: High dose only Acinetobacter spp.: High dose only Staphylococcus spp.: High dose only Gonorrhoea: 0.5 g oral as a single dose</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.5 g x 1 oral or 0.5 g x 1 iv</td>
<td>0.5 g x 2 oral or 0.5 g x 2 iv</td>
<td>Pseudomonas spp.: High dose only Acinetobacter spp.: High dose only S. pneumoniae: High dose only</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.4 g x 1 oral or 0.4 g x 1 iv</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>0.4 g x 2 oral</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>0.2 g x 2 oral or 0.2 g x 2 iv</td>
<td>0.4 g x 2 oral or 0.4 g x 2 iv</td>
<td>Staphylococcus spp.: High dose only</td>
</tr>
</tbody>
</table>

EUCAST – dosing and administration of antibiotics and the relationship to breakpoints.

Gunnar Kahlmeter, EUCAST 2019
Terminology in speech and writing

- Report the bacterium S, I or R (laboratory report)
- The isolate is S, I or R to the agent in question.
- The isolate belongs to the S, I or R category.
- The isolate is categorised as susceptible at normal dosing, susceptible at increased exposure and resistant.
- The isolate can be called susceptible or resistant (but not intermediate)
- Isolates which test S or I are called susceptible.

- ”Non-susceptible”, which was used to describe isolates which were I or R, now only describes resistant isolates.

Gunnar Kahlmeter, EUCAST 2019
Consequences of the new S, I and R......
in the clinic.

• Agents with an “I” in the laboratory report must be considered a therapeutic alternative – some will never be “S”.
• The need for increased exposure must be considered.
• A number of wild type populations, hitherto categorised as “Susceptible” (but with a note in tables that high dose is required) will be re-categorised “I” (Susceptible, increased exposure).
  – Some of these are already implemented in v 9.0, 2019.
    • Examples Acinetobacter vs. fluoroquinolones; Proteii vs. imipenem.
  – Others are under development and will appear during 2019 for consultation.
Consequences of the new S, I and R..... in the laboratory.

"I" as a methodological buffer is gone.

The laboratory can no longer “hide” behind an “intermediate” – you need to get it right and QC your results, irrespective of what method you are on.

It also places major responsibility on manufacturers of devices, panels, disks, agar, to make sure their products and material are up to speed and can be quality controlled in daily microbiology.
Original article

The quality of antimicrobial discs from nine manufacturers—EUCAST evaluations in 2014 and 2017

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Tuesday 21.30

EUCAST evaluation of 21 Mueller Hinton powders for disk diffusion testing - wide differences detected

Jenny Åhman¹, Erika Matuschek¹, Anna-Karin Wallgren¹ and Gunnar Kahlmeter¹

¹EUCAST Development Laboratory, Växjö, Sweden

Clinical Microbiology and Infection
A logical progression...

• In 2019 year’s table, there are a number of S-breakpoints qualified with the superscript ”HE” (for high exposure).

• During 2019 we will consult the world at large on the following proposal.....the logical progression of the new definitions.
In several instances there will only be.....

I and R

....for some organisms and agents there will never be "S".
<table>
<thead>
<tr>
<th>Species</th>
<th>Agent</th>
<th>Current breakpoint</th>
<th>Proposed new breakpoint</th>
<th>From &quot;S&quot; to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacterales</td>
<td>Cefuroxime IV</td>
<td>8/8&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/8</td>
<td>I</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>Piperacillin-tazo</td>
<td>16/16&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/8</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Ticarcillin</td>
<td>16/16&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/8</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime</td>
<td>8/8&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/8</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Aztreonam</td>
<td>16/16&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/16</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>0.5/0.5&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/0.5</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Levofloxacin</td>
<td>1/1&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/1</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Imipenem</td>
<td>4/4&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/4</td>
<td>I</td>
</tr>
</tbody>
</table>

Preliminary, decision and consultation pending
<table>
<thead>
<tr>
<th>Species</th>
<th>Agent</th>
<th>Current breakpoint</th>
<th>Proposed new breakpoint</th>
<th>From &quot;S&quot; to</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. maltophilia</em></td>
<td>Trimethoprim-sulfamethoxazole</td>
<td>4/4</td>
<td>0.001/2</td>
<td>I</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>Ciprofloxacin</td>
<td>1/1&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/1</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Levofloxacin</td>
<td>1/1&lt;sup&gt;HE&lt;/sup&gt;</td>
<td>0.001/1</td>
<td>I</td>
</tr>
<tr>
<td><em>Strc. A,C,G</em></td>
<td>Levofloxacin</td>
<td>2/2</td>
<td>0.001/2</td>
<td>I</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>Levofloxacin</td>
<td>2/2</td>
<td>0.001/2</td>
<td>I</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>Amoxicillin (oral)</td>
<td>2/2</td>
<td>0.001/2</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Amoxi-clav (oral)</td>
<td>2/2</td>
<td>0.001/2</td>
<td>I</td>
</tr>
</tbody>
</table>

**Preliminary, decision and consultation pending**
ATU

The Area of Technical Uncertainty
Most testing AST is unproblematic

...if your method is robust
...if your device is QC:ed
...if you trust and QC your gradient test
...if your disks are high quality
...if your MH medium is dependable
...if the agent and the species cooperate
Cefoxitin 30 μg vs. MIC
S. aureus, 287 isolates (348 correlates)
(7 data sources)

Breakpoints
MIC (screen)  S≤4, R>4 mg/L
Zone diameter (screen)  S≥22, R<22 mm
ECOFF  4 mg/L
Cefoxitin 30 µg vs. mecA status
*S. aureus*, 170 isolates
(3 data sources)

**Breakpoints**
Zone diameter (screen)  
S≥22, R<22 mm
Variability in MIC and disk testing

Trained professionals with good quality material can 90-95% of the time attain:

- a target MIC value +/- 1 dilution
- a target zone diameter +/- 2 mm
Repeat MIC testing using broth quality controlled brothmicro trays.

The best you can achieve!

Almost all results on target +/- 1 MIC dilution.
Piperacillin-tazobactam / Escherichia coli ATCC 25922
International wild type zone diameter distribution - Reference database 2019-04-10
EUCAST disk diffusion method

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

Zone diameter (mm)

Cefoxitin / Staphylococcus aureus ATCC 29213
International wild type zone diameter distribution - Reference database 2019-04-10
EUCAST disk diffusion method

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

Zone diameter (mm)
Meropenem and Enterobacterales – one of many examples where an ATU is not needed.
Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints for uncomplicated UTI

Amoxicillin-clavulanic acid 20-10 µg vs MIC
Enterobacterales, 325 isolates

MIC with fixed concentration of clavulanic acid at 2 mg/L

Breakpoints (uncomplicated UTI)
MIC S≤32, R>32 mg/L
Zone S≥16, R<16 mm

ATU not needed

Gunnar Kahlmeter, EUCAST 2019
BUT, sometimes there is a need for a “warning”

• Variation in the method which is difficult to control.
• Variation in the interpretation which is difficult to control
  – Breakpoint splits wild type (mostly avoided by EUCAST)
  – Breakpoint splits an important resistant population (ceftaroline in MRSA)
Area of Technical Uncertainty (ATU)

- ATU is not a fourth susceptibility category – it is to warn laboratory staff about an area where interpretation is difficult.

- The ATU is not to compensate for poor methodological skills – on the contrary, AST today require more skills than ever before.

- ATU is defined by a single MIC-value or a short range of zone diameter values.

- How the ATU is dealt with depends on the situation (the sample, the agent, the infecting organism).
Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints for systemic infections

Amoxicillin-clavulanic acid 20-10 µg vs MIC
Enterobacterales, 325 isolates

MIC with fixed concentration of clavulanic acid at 2 mg/L

Breakpoints (systemic infections)
- MIC: S≤8, R>8 mg/L
- Zon: S≥19, R<19 mm

ATU 19-20 mm
Ceftaroline 5 μg vs. MIC
S. aureus, 216 isolates (593 correlates)

ATU 1 mg/L, 19-20 mm

Breakpoints (pneumonia)
MIC S≤1, R>1mg/L
Zone diameter S≥20, R<20 mm
Piperacillin-tazobactam 30-6 μg vs. MIC
Enterobacterales, 531 isolates (840 correlates)

Breakpoints
MIC
S≤8, R>16 mg/L
Zone diameter
S≥20, R<17 mm

ATU 16 mg/L
17 – 19 mm
E. coli and K. pneumoniae from the Merino trial
Courtesy Andrew Henderson, Brisbane, Australia
Current ATUs (2019)

• Enterobacterales  amoxicillin-clavulanic acid (systemic)
  piperacillin-tazobactam
  ciprofloxacin

• Ps. aeruginosa  piperacillin-tazobactam
  ceftazidime-avibactam

• S. aureus  ceftaroline, ceftobiprole

• S. epidermidis  MRSE cefoxitin screen test

• H. influenzae with PBP3-mutations (beta lactams)
### EUCAST breakpoint table v.9.0 (2019) with columns for ATU

<table>
<thead>
<tr>
<th>Penicillins¹</th>
<th>MIC breakpoint (mg/L)</th>
<th>Disk content (µg)</th>
<th>Zone diameter breakpoint (mm)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzylpenicillin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>8, 8</td>
<td>10</td>
<td>14, 14</td>
<td></td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>8, 8</td>
<td>10-10</td>
<td>14, 14</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>8, 8</td>
<td>-</td>
<td>Note, Note</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>8, 8</td>
<td>20-10</td>
<td>19, 19, 19-20</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid (uncomplicated UTI only)</td>
<td>32, 32</td>
<td>20-10</td>
<td>16, 16, 16</td>
<td></td>
</tr>
<tr>
<td>Piperacillin</td>
<td>8</td>
<td>16</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>8, 16</td>
<td>16, 30</td>
<td>20, 17</td>
<td>B</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>8</td>
<td>16</td>
<td>23</td>
<td>C</td>
</tr>
<tr>
<td>Ticarcillin-clavulanic acid</td>
<td>8, 16</td>
<td>16, 75-10</td>
<td>23</td>
<td>D</td>
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<tr>
<td>Temocillin</td>
<td>Note</td>
<td>Note</td>
<td>Note</td>
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<tr>
<td>Phenoxyacillin</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Oxacillin</td>
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<td>-</td>
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<tr>
<td>Cloxacillin</td>
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<td>-</td>
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<tr>
<td>Dicloxacillin</td>
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<tr>
<td>Flucloxacillin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

| Mecillinam (uncomplicated UTI only) E. coli, Klebsiella spp. (except K. aerogenes), Raoultella spp. and P. mirabilis | 8, 8 | 10 | 15, 15 |       |

**Notes**

- Numbered notes relate to general comments and/or MIC breakpoints.
- Lettered notes relate to the disk diffusion method.

1/A. Wild type Enterobacterales are categorised as susceptible to aminopenicillins. Some countries prefer to categorise wild type isolates of *E. coli* and *P. mirabilis* as “Susceptible, increased exposure”. When this is the case, use the MIC breakpoint $S \leq 0.5$ mg/L and the corresponding zone diameter breakpoint $S \geq 50$ mm.

2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.

3. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.

4. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.

5. Breakpoints still under consideration.

6. Agar dilution is the reference method for mecillinam MIC determination.

B. Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars.

C. Susceptibility inferred from ampicillin.

D. Ignore isolated colonies within the inhibition zone for *E. coli*. 

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Gunnar Kahlmeter, EUCAST 2019
ATU –alternative actions

• **Repeat the test** – if test failed technically.
• **Confirm** using an alternative test (MIC, PCR, PBP-agglutination...).
• **Report the result with comment** – “uncertain result”.
• **Down-grade interpretation**: S to I, I to R.
• **Discuss and explain** to clinical colleagues.
Try hard...

• IF only few alternative antibiotics for therapy.
• IF in a positive blood culture (or other serious infection).
• IF it can be easily solved.

• BUT if there are many alternatives, THEN report blank and add a comment.
Thank you!

gunnar.kahlmeter@eucast.org
3. RAST – Rapid AST directly from blood culture bottles

Emma Jonasson, Erika Matuschek, Martin Sundqvist, Anna Åkerlund, Gunnar Kahlmeter

On behalf of EUCAST
**The European Committee on Antimicrobial Susceptibility Testing - EUCAST**

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. EUCAST was formed in 1997. It has been chaired by Ian Phillips (1997 - 2001), Gunnar Kahlmeter (2001 - 2012), Rafael Canton (2012 - 2016) and Christian Giske (2016 -). Its scientific secretary is Derek Brown (1997 - 2016) and John Turnidge (2016 -). Its webmaster is Gunnar Kahlmeter (2001 -). From 2016, Rafael Canton is the Clinical Data Co-ordinator and Gunnar Kahlmeter the Technical Data Co-ordinator.

EUCAST deals with breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing and functions as the breakpoint committee of EMA and ECDC. EUCAST does not deal with antibiotic policies, surveillance or containment of resistance or infection control. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European and other countries, FESCI and ISC. The Steering Committee also consults on EUCAST proposals with experts within the fields of infectious diseases and microbiology, pharmaceutical companies and susceptibility testing device manufacturers.

EUCAST has several subcommittees - [see page Subcommittees.](#)

Antimicrobial MIC breakpoints in Europe have been harmonised by EUCAST and since a few years all European countries now follow EUCAST guidelines. Many countries outside Europe have adopted or adapted EUCAST breakpoints.
Rapid AST directly from blood culture bottles

EUCAST will shortly publish recommendations for short incubation (4, 6 and 8 hours) AST directly from positive blood culture bottles using EUCAST standard disk diffusion. These are the characteristics of the rapid method:

- direct inoculation of disk diffusion plates (MH, MH-F) using 100 - 150 µL directly from a positive blood culture bottle (BD, bioMérieux and Thermo Fisher).
- no centrifugation or dilution of the inoculum - inoculate plates as for standard EUCAST disk diffusion.
- shortened incubation - 4, 6 and 8 hours with breakpoints adapted to each incubation time.
- breakpoints for each species and each reading time.
- identity of species must be known prior to interpretation of AST results.
- the method is currently validated for the following species:
  - Escherichia coli
  - Klebsiella pneumoniae
  - Pseudomonas aeruginosa
  - Staphylococcus aureus
  - Streptococcus pneumoniae
  - Enterococcus faecalis and Enterococcus faecium
- a positive blood culture bottle should be processed 0 - 18 hours after the positive signal.
- zone diameters are read from the front of the plate after removal of the lid.
- not all zone diameters can be read after 4 or 6 hours.
- read zone diameters ONLY when an obvious zone edge can be identified - otherwise reincubate and read after 6 or 8 hours.
- the breakpoint table is specific for EUCAST Rapid AST - do not use the regular breakpoint table. Each species has its own TAB in the table and each reading time (4, 6 and 8 hours) its own section.
Snabb resistensbestämning direkt från blododlingsflaskor

- Art-ID på 60 min (87%)
- Resistensbestämning inom 4, 6 eller 8 timmar.
- 100 – 150 uL direkt från blododlingsflaskan till MH/MHF-medier
- Omedelbar inkubation och avläsning efter 4, 6 och 8 timmar.
- Rapportering: endast S och R
- Lämna blankt om i ATU eller zonen ej tydligt läsbar. - inkubera omedelbart igen och läs efter 6 och 8 timmar.
- Om osäkert/inget resultat efter 8 timmar – utför standardiserad 16 – 20 h resistensbestämning.
- Flödet på laboratoriet avgör den diagnostiska gången och möjligheterna.

Gunnar Kahlmeter, EUCAST 2019
Species

The method is currently validated for the following species.

– *Escherichia coli*
– *Klebsiella pneumoniae*
– *Pseudomonas aeruginosa* (6, 8h)
– *Staphylococcus aureus*
– *Streptococcus pneumoniae*
– *Enterococcus faecalis* and *Enterococcus faecium*

• *Acinetobacter*, *S.epidermidis* ....
• More antibiotics...
**Escherichia coli**

Zone diameter breakpoints for RAST directly from blood culture bottles

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Disk content (µg)</th>
<th>4 hours</th>
<th>6 hours</th>
<th>8 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S ≥</td>
<td>ATU</td>
<td>R &lt;</td>
<td>S ≥</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>30-6</td>
<td>17</td>
<td>12-16</td>
<td>12</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>5</td>
<td>15</td>
<td>13-14</td>
<td>13</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>10</td>
<td>15</td>
<td>12-14</td>
<td>12</td>
</tr>
<tr>
<td>Meropenem</td>
<td>10</td>
<td>18</td>
<td>15-17</td>
<td>15</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5</td>
<td>17</td>
<td>14-16</td>
<td>14</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
<td>15</td>
<td>13-14</td>
<td>13</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>14</td>
<td>12-13</td>
<td>12</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>10</td>
<td>14</td>
<td>12-13</td>
<td>12</td>
</tr>
</tbody>
</table>

Notes
1. Screening breakpoints for ESBL or carbapenemase production have not yet been validated. The breakpoints listed are clinical breakpoints. Isolates that are resistant or in the ATU may be suspected of having beta-lactamase mediated resistance.
"Blank results"

Laboratoriet bör överväga att inkludera en kommentar som förklarar varför visa resultat lämnats blanka i resistensbeskedet:

"Resistensbestämning med tidig avläsning av resultat (4, 6 eller 8 timmar) förutsätter att endast pålitliga resultat rapporteras. Ett blankt resultat i en rapport kan följas av ett pålitligt resultat i en senare avläsning."

Gunnar Kahlmeter, EUCAST 2019
The EUCAST RAST clinical breakpoint are based on data from three studies.

1. **Spiked bottles with selected difficult isolates**, performed at EDL. Isolates have been tested with the RAST method on MH-agar from Oxoid and BD/BBL. Reference method was BMD.

2. **Clinical trial northern Europe**, clinical isolates from 40 laboratories. Locally used MH-agars and antimicrobial discs. Reference method is EUCAST disk diffusion 16-20 h (validated against BMD).

3. **Clinical trial southern Europe**, clinical isolates from 15 laboratories. Locally used MH-agar and antimicrobial discs. Reference method is EUCAST disk diffusion 16-20 h.

Gunnar Kahlmeter, EUCAST 2019
### Blood culture bottles

- Bactec
- BactAlert (old and new)
- VersaTREK

### Media

- Oxoid (Thermo Fisher)
- BBL (BD)
- Agricon Ricerche
- bioMérieux
- Bio-Rad
- Liofilchem
- LIP/Fannin

### Disks

- BD
- Bio-Rad
- I2A
- MAST
- BioMaxima
- Oxoid
- Rosco
Clinical trials
Ceftobiprole 5 µg vs. MIC
S. aureus, 114 isolates (228 correlates)

ATU 2 mg/L, 16-17 mm

Breakpoints
MIC
S ≤ 2, R > 2 mg/L
Zone diameter
S ≥ 17, R ≤ 17 mm
Current (2019) ATU warnings:

<table>
<thead>
<tr>
<th>#</th>
<th>Species</th>
<th>Agent</th>
<th>Breakpoints ≤/&gt; (MIC)</th>
<th>ATU</th>
<th>Breakpoints ≥/&lt; (zone)</th>
<th>ATU</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Enterobacterales</em></td>
<td>Amoxicillin-clavulanic</td>
<td>8/8</td>
<td>-</td>
<td>19/19</td>
<td>19-20</td>
</tr>
<tr>
<td>2</td>
<td><em>Enterobacterales</em></td>
<td>Piperacillin-tazobactam</td>
<td>8/16</td>
<td>16</td>
<td>20/17</td>
<td>17 - 19</td>
</tr>
<tr>
<td>3</td>
<td><em>Enterobacterales</em></td>
<td>Ceftaroline</td>
<td>0.5/0.5</td>
<td>-</td>
<td>23/23</td>
<td>22 - 23</td>
</tr>
<tr>
<td>4</td>
<td><em>Enterobacterales</em></td>
<td>Ciprofloxacin</td>
<td>0.25/0.5</td>
<td>0.5</td>
<td>25/22</td>
<td>22 - 24</td>
</tr>
<tr>
<td>5</td>
<td><em>Pseudomonas</em></td>
<td>Piperacillin-tazobactam</td>
<td>16/16</td>
<td>-</td>
<td>18/18</td>
<td>18 - 19</td>
</tr>
<tr>
<td>6</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Ceftazidime-avibactam</td>
<td>8/8</td>
<td>17/17</td>
<td>16 - 17</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Colistin</td>
<td>2/2</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td><em>S. epidermidis</em></td>
<td>Cefoxitin-screen</td>
<td>-</td>
<td>-</td>
<td>25/25</td>
<td>25 - 27</td>
</tr>
<tr>
<td>9</td>
<td><em>S. aureus</em></td>
<td>Ceftaroline</td>
<td>1/1</td>
<td>1</td>
<td>20/20</td>
<td>19 - 20</td>
</tr>
<tr>
<td>10</td>
<td><em>S. aureus</em></td>
<td>Ceftriaxone</td>
<td>2/2</td>
<td>2</td>
<td>17/17</td>
<td>16 - 17</td>
</tr>
<tr>
<td>11</td>
<td><em>S. aureus</em></td>
<td>Amikacin</td>
<td>8/16</td>
<td>16</td>
<td>18/16</td>
<td>15 - 19</td>
</tr>
<tr>
<td>12</td>
<td><em>H. influenzae</em> (PBP3 mutation)</td>
<td>Ampicillin</td>
<td>1/1</td>
<td>-</td>
<td>16/16</td>
<td>16 - 19</td>
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<tr>
<td>13</td>
<td><em>H. influenzae</em> (PBP3 mutation)</td>
<td>Amoxicillin-clavulanic</td>
<td>2/2</td>
<td>-</td>
<td>15/15</td>
<td>14 - 16</td>
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<td>14</td>
<td><em>H. influenzae</em> (PBP3 mutation)</td>
<td>Piperacillin-tazobactam</td>
<td>0.25/0.25</td>
<td>-</td>
<td>27/27</td>
<td>24 - 27</td>
</tr>
<tr>
<td>15</td>
<td><em>H. influenzae</em> (PBP3 mutation)</td>
<td>Several cephalosporins</td>
<td>See breakpoints</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td><em>H. influenzae</em> (PBP3 mutation)</td>
<td>Imipenem</td>
<td>2/2</td>
<td>-</td>
<td>20/20</td>
<td>6 - 19</td>
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