Educational Workshop

EW01: Antimicrobial susceptibility testing
arranged with the European Committee on Antimicrobial Susceptibility Testing (EUCAST)

Convenors: Gunnar Kahlmeter (Vaxjo, SE)
Derek Brown (Peterborough, UK)

Faculty: Derek Brown (Peterborough, UK)
Gunnar Kahlmeter (Vaxjo, SE)
Johan Mouton (Nijmegen, NL)
Alasdair MacGowan (Bristol, UK; no presentation submitted)
Rafael Canton (Madrid, ES)
EUCAST definitions
(and breakpoint table, MIC and zone distribution website conventions)

Derek Brown

ECCMID 2011, Milan

Breakpoints and cut-off values

• Clinical breakpoints

• Non-species related breakpoints

• Epidemiological cut-off value (ECOFF)

Clinical breakpoints

• Values that correlate with clinical outcome

• MICs are the primary breakpoints

• Breakpoints in other methods are related to MIC breakpoints
Brown - EUCAST definitions

Clinically susceptible

A microorganism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success.

- A microorganism is categorized as susceptible (S) by applying the appropriate breakpoint in a defined phenotypic test system.
- This breakpoint may be altered with legitimate changes in circumstances.

Clinically resistant

A microorganism is defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure.

- A microorganism is categorized as susceptible (S) by applying the appropriate breakpoint in a defined phenotypic test system.
- This breakpoint may be altered with legitimate changes in circumstances.

Clinically intermediate

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 physically 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.
Non-species-related breakpoints

- Pk/Pd (pharmacokinetics/pharmacodynamic) breakpoints
- Calculated by statistical techniques (e.g., Monte Carlo simulation) to estimate the probability of attaining the target value of the relevant Pk/Pd index for microorganisms with different MICs
- Used in clinical breakpoint development and in situations where a breakpoint may be required but there is no species-specific breakpoint
- Clinical breakpoints will be same unless they split the wild type or there is no resistance

Epidemiological cut-off value (ECOFF)

- MIC value identifying the upper limit of the wild type population

A microorganism is defined as wild type (WT) for a species by the absence of acquired and mutational mechanisms of resistance to the agent.
Epidemiological cut-off values (ECOFFs)

• Estimated by visual inspection or statistically calculated (Turnidge et al CMI 2006;12:418-25)
• The ECOFF is not changed by sampling time, source (human, animal, environmental), geographical origin
• Used in clinical breakpoint development and as a sensitive indicator of resistance development in surveillance studies.

There is no definite relationship between the ECOFF and the clinical breakpoint

The ECOFF may be higher, the same, lower than a clinical breakpoint
Brown - EUCAST definitions

Clinical breakpoint higher than ECOFF

Clinical breakpoint same as ECOFF

Clinical breakpoint lower than ECOFF
**EUCAST breakpoint format**

- **Susceptible**
  - \( S \leq 1 \)
  - **Resistant**
  - \( R > 4 \) mg/L

- Zone \( R < 24 \text{mm} \) \( \equiv \) \( \leq \) 23 mm

**EUCAST intermediate category**

- Intermediate category is not displayed e.g. Ciprofloxacin
  - \( S \leq 0.5 \text{mg/L} \), \( R > 1 \text{mg/L} \)
  - Intermediate inferred \( \geq 0.5\text{-}1 \text{mg/L} \)

- \( S \geq 22 \text{mm mg/L} \), \( R < 18 \text{mm} \)
  - Intermediate inferred \( 18\text{-}21 \text{mm} \)

**EUCAST table abbreviations**

- **"-"** Susceptibility testing not recommended as the species is a poor target for therapy with the drug (do not test, or do not report, or report as R without testing)

- **"IE"** Insufficient evidence to set a breakpoint (report MIC values with comment but no categorical interpretation)
"NA" Not Applicable (mostly used for screening tests when they are not applicable to particular organisms)

"IP" In preparation (breakpoints will be established)

EUCAST breakpoint table links to rationale documents

EUCAST breakpoint table links to MIC distributions
EUCAST MIC and zone distributions

Select MIC or zone
diameter distribution
Select agent or
species
Blue indicates
wild type
Clinical breakpoints
and ECOFF
Link to
graph

EUCAST MIC distributions

Do not infer resistance rates

EUCAST MIC distributions
Insufficient data to define ECOFF
Summary

• EUCAST provides clinical breakpoints, non-species related breakpoints and epidemiological cut-off values (ECOFF)
• Be aware of formatting conventions and abbreviations in EUCAST tables and on EUCAST MIC and zone distributions website

www.EUCAST.org
An MIC is an MIC is an MIC, isn’t it?

Gunnar Kahlmeter
Clinical microbiology
Växjö, Sweden

• MIC – the minimum inhibitory concentration (mg/L or µg/mL)

• The lowest concentration in a series of twofold concentrations that will inhibit the growth of a microorganism, as measured by the naked eye.

• Convention: The series of concentrations shall contain the concentration 1 mg/L.
Kahlmeter - A MIC is a MIC is a MIC – isn’t it?

MIC
in vitro
relative

If you perform MIC determinations on many isolates of a well defined species using a standardized method, this is what you get:
3615 MICs from 11 data sources

...or this:
87 764 MICs from 33 data sources
Kahlmeter - A MIC is a MIC is a MIC – isn’t it?

...or this 29,049 MICs from 13 data sources

....a distribution of MICs covering 3 – 5 dilution steps in a series of two-fold dilutions.

Methods for MIC determination

• Broth dilution
• Broth microdilution*
• Agar dilution
• Gradient tests

*ISO standard for non-fastidious microorganisms
Features of MIC dilution methods

<table>
<thead>
<tr>
<th></th>
<th>Broth MIC</th>
<th>Agar MIC</th>
<th>Gradient test</th>
<th>Disk diffusion</th>
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<td>Contamination</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>detected</td>
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<td>Inoculum effect</td>
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<td>- beta-lactamase</td>
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<td>Useful for slow</td>
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<td>+++</td>
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<td>Direct AST</td>
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<tr>
<td>Automation</td>
<td>Yes</td>
<td>Partial</td>
<td>No</td>
<td>(Partial)</td>
</tr>
</tbody>
</table>

Methods used in automated systems

- **Broth microdilution**
  - Limited dilution series
    - No easy extension to embrace changes in breakpoints
    - Two concentrations only – S and R breakpoint
  - Growth characteristics in the presence and absence of antibiotics at varying but few concentrations
    - Not true MIC-values
  - “black box” results

- **Gradient tests** – semiautomated ??
- **Disk diffusion** – semiautomated ??

ISO 20776-1 (2006)

- **Title:** “Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices”
  - **Part 1:** “Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases”.
  - **Broth microdilution technique.**
  - Rapidly growing aerobic bacteria involved in infectious diseases.
  - 2.5-5% lysed horse blood for *Streptococcus* spp.
  - No recommendations for Haemophilus, anaerobes or other fastidious organisms.
Kahlmeter - A MIC is a MIC is a MIC – isn’t it?

**E. coli vs. several antibiotics**

Broth microdilution

International standard for evaluating other susceptibility testing methods

  - **Title:** Clinical laboratory testing and *in vitro* diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices.
  - **Part 2:** Evaluation of performance of antimicrobial susceptibility test devices
  - For comparison of other MIC methods, automated systems and disk diffusion with the ISO broth microdilution method

Is there a true (reproducible, useful) difference between individuals in the wild type MIC distribution?
Kahlmeter - A MIC is a MIC is a MIC – isn’t it?

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 on the bug
  - method, pH, cations, incubation atmosphere

The width of the MIC (or zone diameter) distribution:
- Variations in exogenous factors that influence the activity of the drug
  (pH, cations, incubation atmosphere and time, etc)
- Variations in endogenous factors among individual organisms that
  influence the activity of the drug
  - any biological characteristic such as generation time, nutrient
  dependency, atmosphere dependency etc
- The degree of standardisation of method used
- The number of investigators & observations
- The stability of the molecule
- ...

E. coli wild type; generation times (min) (n=100)

An example of biological variation not primarily
related to antibiotic susceptibility.

The EUCAST MIC distributions show "% organisms" and bars
when "number of measurements are ≥1% of total".

Very similar S. aureus
vancomycin MIC
distributions from 33
sources using various
MIC methods.
Kahlmeter - A MIC is a MIC is a MIC – isn’t it?

The EUCAST MIC data if presented as "numbers" (not per cent) and with bars for all data.

Vancomycin / Staphylococcus aureus (n=87 764)
EUCAST MIC distribution – Reference database 2011-04-21

The EUCAST MIC data if presented as "numbers" (not per cent) and with bars for all data.

Calibration

MICs performed with different methods are often surprisingly similar!

- methods are often tightly calibrated against each other
- the degree of calibration at low end (wild type organisms) and high end (organisms with resistance mechanisms) may differ and resistance mechanisms may affect different methods to varying extent.
Kahlmeter - A MIC is a MIC is a MIC – isn’t it?

Standardisation

• If you provide the world at large with a not too complicated recipe and all the ingredients, it is not surprising that Caesar salad is Ceasar salad is Caesar salad

…or an MIC is an MIC is an MIC…

"…albeit with a twist!"

"…and not necessarily the only salad!"

MIC reproducibility

A well standardised method can provide MICs at

 +/- 1 dilution step 95% of time
  +/- 2 dilution step 99 % of time

Spread of *S. maltophilia* ciprofloxacin MIC distributions from 15 sources using various MIC methods
Kahlmeter - A MIC is a MIC is a MIC – isn’t it?

Cefuroxime vs. E.coli

Ciprofloxacin

S.pneumoniae vs ciprofloxacin
Variables that may affect MICs in broth dilution tests

- **Medium (type, brand, batch)**
  - all antibiotics and microorganisms
- **Incubation time**
  - ...the longer the higher the MIC...
- **Incubation temperature**
  - affects growth, antibiotic activity, expression of resistance mechanism, diffusion
- **Inoculum – size**
  - The larger the inoculum, the higher the MIC
- **Inoculum – growth phase**
  - the condition of the organisms affects the lag phase – the longer the lag phase, the lower the MIC
- **Atmosphere**
  - examples: CO₂ affects pH which affects macrolides; anaerobic atmosphere results in lower metronidazole MICs for *Helicobacter pylori*
- **pH**
  - Some antibiotics are more active in alkaline and some in acid environment: examples to follow.
- **Ion content**
  - Aminoglycosides are affected by Ca²⁺, Mg²⁺ and daptomycin by Ca²⁺
- **Reading problems**
  - Trailing endpoints (Acinetobacter vs. betalactams)

MIC – in summary

**Pros**
- Basis for all phenotypic susceptibility testing of bacteria and fungi
- Quantifiable
- Predicts susceptibility and resistance
- Can be highly standardized for most antibiotics and species

**Cons**
- In vitro
- Relative
- Requires tight standardisation
Johan W. Mouton MD PhD FIDSA

PK/PD Tools for setting breakpoints

Johan Mouton - PK-PD tools for breakpoints definition

LAB REPORT

MIC testing

- Provides Clinician/Consultant guidelines how to optimally treat a patient (Freely translated from EUCAST guideline)
Johan Mouton - PK-PD tools for breakpoints definition

**MIC**
Measure of Potency

**MIC**
Lowest concentration with no visible growth after 18 hour incubation

\[ \begin{array}{cccccc}
0.25 & 0.5 & 1 & 2 & 4 & 8 \\
\bullet & \bullet & \bullet & \circ & \circ & \circ \\
\end{array} \]

\[ \text{MIC} = 2 \text{ mg/L} \]

**Efficacy of the drug**

Potency of a drug (MIC)  
Exposure to the bug (PK)

**Antimicrobial Efficacy of the Drug**
(Microbiological Cure)

Effect on Host  
(Clinical Cure)
**Pharmacokinetic parameters:**

*Measures of Exposure*

\[ \text{AUC is usually linearly related to Dose} \]

- AUC

**Pharmacokinetic Parameter (and Dose)**

- MIC

Thus, we have to:
- Establish a relationship between the MIC in vitro and concentrations in vivo (thus, dosing regimens)
- Determine MICS that can be covered by the dosing regimen used
- Determine the highest MIC that can be covered for all patients, given the dose

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**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.

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**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.
Any idea where we are today?

No idea…

May be a mouse?

Might be a human, though…

No idea…

…

May be a mouse?

Might be a human, though…

An elephant….

Today it is an elephant!

THE TARGET IS THE MICRO-ORGANISM
Johan Mouton - PK-PD tools for breakpoints definition

Neutropenic Mouse Thigh-Infection Model

1. Neutropenia induced by 2 injections of cyclophosphamide on days -4 and -1
2. Bacteria injected into thighs on day 0 (10^6)
3. Treatment (usually given SQ) started 2 hr after infection and continued for 1-5 days
4. Thighs removed, homogenized, serially diluted and plated for CFU determinations

Fluconazole efficacy in mice
Dose vs MIC

'Normalizing pk/pd relationships'
Pharmacokinetic parameter  MIC
Pharmacodynamic index (AUC/MIC, Peak/MIC, T>MIC)
Johan Mouton - PK-PD tools for breakpoints definition

Fluconazole Pharmacodynamics Against Isogenic Strain Pairs of Susceptible and Resistant C. albicans

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

Pharmacodynamic index
(AUC/MIC)

Rodriguez-Tudela et al, AAC 2007

Relationships Between 24-Hr fAUC/MIC and Efficacy against Pneumococci for Fluoroquinolones in Animals

- A clear relationship exists between exposure and effect
- A maximum effect is reached at ratio's of 25-35 (mortality)

Relationship between faUC/MIC and Effect
121 patients with S. pneumoniae respiratory infection

- Relationship between AUC: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.

Quantitative relationship: exposure in mice and men

AUC/MIC mouse
AUC/MIC human

~ 30-35

Efficacy of an antimicrobial

Animal
Correlation Treatment - Effect
Outcome parameter

Human
Correlation Treatment - Effect
Outcome parameter

Qualitative relationship (pk/pd index)
Quantitative relationship (value pk/pd index)
Here, we observe that the pk/pd Index (NOT dose!!!) matches
- qualitatively
- quantitatively

Relationship between \( \text{fAUC/MIC} \) and Effect
121 patients with S. pneumoniae respiratory infection

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

Clearly, there is a direct relationship between pk/pd index (AUC/MIC) and effect
higher values are associated with a higher likelihood of therapeutic success
Johan Mouton - PK-PD tools for breakpoints definition

**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

Pharmacodynamic index
(AUC/MIC)

Rodriguez-Tudela et al, AAC 2007

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**It is not only for Mice**

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Exposure model</th>
<th>AUC/MIC target (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole</td>
<td>Micronidazole</td>
<td>600-1200 mg/l/day</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Micronidazole</td>
<td>400-800 mg/l/day</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>Micronidazole</td>
<td>20-40 mg/l/day</td>
</tr>
</tbody>
</table>

Rodriguez-Tudela et al, AAC 2007

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**SETTING A BREAKPOINT –PK/PD**

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

ESTIMATE EXPOSURE  from the dosing regimen and PK

CALCULATE BREAKPOINT  from PK/PD target = PK/PD Index

Ambrose et al, CID 2007

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32
Be sure that the \( \frac{fAUC}{MIC} \) ratio is at least appr. 34 in every patient.

**Clinical practice:**
When starting treatment, we do not know:
- the AUC in the individual patient.

**Levofloxacin 500 mg**
\( fAUC = 30-50 \text{ mg/L} \)
Pharmacokinetics

Some people are more equal than others…

fAUC distribution levofloxacin
(monte carlo simulation)
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.

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

- 95% percentile
- 99% percentile
- Mean

JWM Milan 07-05-11
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.

JWM Milan 07-05-11

Mouton et al., 2004
High dose levofloxacin
2x 500 mg, or 750 mg
AUC 70-80

S = 0.5 mg/L

"He chose poorly"
Knight from Indiana Jones:
The Last Crusade
Johan Mouton - PK-PD tools for breakpoints definition

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

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

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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.

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WWW.EUCAST.ORG
Rationale for new or revised EUCAST clinical breakpoints

Derek Brown

ECCMID 2011, Milan

Setting breakpoints in EUCAST

• Harmonization of European breakpoints
• Setting breakpoints for new agents (with EMA)
• Review of established breakpoints

EUCAST procedure for harmonizing breakpoints

• Collect and evaluate data in Steering Committee
• Consult on proposed breakpoints
• Decision and publication with rationale

Breakpoints for all more widely used agents have been reviewed and harmonized breakpoints agreed
Some less common agents and organisms currently under review
Setting breakpoints for new agents

- Pharmaceutical company submits new agent to European Medicines Agency (EMA) for marketing approval
- Relevant data are shared with the EUCAST Steering Committee (confidential process)
- EMA approves (or not) clinical indications, dosages, administration forms and target organisms
- In consultation with national breakpoint committees EUCAST sets breakpoints for organisms approved by EMA

- Breakpoints set for daptomycin, tigecycline, doripenem and retapamulin (epidemiological cut-off value)
- Currently two agents in process
- Ceftobiprole, garenoxacin, ictaprim, oritavancin withdrawn

Review of breakpoints by EUCAST

- New resistance mechanisms
- New agent in class
- New clinical data
- Extended indications
- Change in dosing or administration
- Change in target organisms

Breakpoints reviewed
Glycopeptides, carbapenems, colistin, cephalosporins, monobactams
Brown - Rationale for new or revised EUCAST clinical breakpoints

EUCAST rationale documents

Dosages in different countries

Introduction

The in vitro response of a group of compounds in a given microorganism may be limited by the matrix in which they are placed. Thus, we describe a list of criteria that will be used by laboratories and will ensure comparability of results between laboratories. These criteria will be described in detail in the EUCAST rationale documents.

Dosages in different countries

<table>
<thead>
<tr>
<th>Drug</th>
<th>EUCAST</th>
<th>CA-MEM</th>
<th>JPS</th>
<th>DIM</th>
<th>INH</th>
<th>RIF</th>
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<tbody>
<tr>
<td>MIC (μg/mL)</td>
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<td>Minimum inhibitory concentration</td>
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<td>Available from</td>
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EUCAST rationale documents

MIC distributions and ECOFFs

MIC distributions and epidemiological cut-off (ECOFF) values (μg/L)

<table>
<thead>
<tr>
<th>Organism</th>
<th>ECOFF (μg/L)</th>
<th>MIC distribution</th>
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<tbody>
<tr>
<td>Acinetobacter baumannii</td>
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<td></td>
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<tr>
<td>Acinetobacter calcoaceticus</td>
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<td>Acinetobacter nosocomialis</td>
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<tr>
<td>Acinetobacter baylyi</td>
<td></td>
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<tr>
<td>Acinetobacter pittii</td>
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</table>
Brown - Rationale for new or revised EUCAST clinical breakpoints

**EUCAST rationale documents**
**Breakpoints prior to harmonization**

<table>
<thead>
<tr>
<th>Species specific compounds</th>
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<tr>
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**EUCAST rationale documents**
**Pharmacokinetics**

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<th>Value (h)</th>
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**EUCAST rationale documents**
**Pharmacodynamics**

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<th>Value (h)</th>
</tr>
</thead>
<tbody>
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<tr>
<td></td>
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</tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

*References*
- European Committee on Antimicrobial Susceptibility Testing (EUCAST). *Pharmacodynamic principles and interpretive criteria for establishing in vitro breakpoints.* 2004

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Brown - Rationale for new or revised EUCAST clinical breakpoints

EUCAST rationale documents
Monte Carlo simulations and Pk/Pd breakpoints

- EUCAST rationale documents
  - Clinical data
    - EUCAST rationale documents
      - Clinical breakpoint summary
        - Non-species-related breakpoints
          - Explains deviations from non-species-related BPs
        - Species without breakpoints, with explanation
        - Clinical qualifications, e.g. UTI only
        - Dosage
        - Additional comments
EUCAST doripenem breakpoints

**Non-species-related breakpoints**

- Pk/Pd and MC analysis indicated non-species-related breakpoints of S≤1, R>4 mg/L

**EUCAST doripenem breakpoints**

<table>
<thead>
<tr>
<th>Species</th>
<th>S≤</th>
<th>R&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Streptococci</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Species without breakpoints**

- *Staphylococcus* spp. Susceptibility inferred from methicillin susceptibility
- *Enterococcus* spp. a poor target
- *N. gonorrhoeae* Insufficient evidence
EUCAST carbapenem breakpoints

- Other carbapenem breakpoints (imipenem, meropenem, ertapenem) reviewed at the same time
- Considered no strong evidence supporting a change from established breakpoints

Revision of EUCAST glycopeptide breakpoints

Original vancomycin breakpoints for *S. aureus* (S ≤ 4 mg/L, R > 8 mg/L) did not distinguish VISA from susceptible isolates.

Old EUCAST vancomycin breakpoints for *Staphylococcus* spp.
Brown - Rationale for new or revised EUCAST clinical breakpoints

EUCAST vancomycin breakpoints

<table>
<thead>
<tr>
<th>Non-species-related breakpoints</th>
<th>Insufficient data to set non-species-related breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staphylococcus spp.</strong></td>
<td>S ≤ R &gt;</td>
</tr>
<tr>
<td><strong>Enterococcus spp.</strong></td>
<td>2 2</td>
</tr>
<tr>
<td><strong>Streptococci</strong></td>
<td>2 2</td>
</tr>
<tr>
<td><strong>Anaerobes (Gm +ve)</strong></td>
<td>2 2</td>
</tr>
</tbody>
</table>

“GISA/VISA” isolates reported resistant - serious infections with “GISA/VISA” isolates are not treatable with increased doses of vancomycin. VanA reported resistant

New EUCAST vancomycin breakpoints for *Staphylococcus* spp.
EUCAST vancomycin breakpoints

<table>
<thead>
<tr>
<th>Species-related breakpoints</th>
<th>S ≤ R &gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus spp.</td>
<td>2</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>2</td>
</tr>
<tr>
<td>Streptococci</td>
<td>2</td>
</tr>
<tr>
<td>Anaerobes (Gm+ve)</td>
<td>2</td>
</tr>
</tbody>
</table>

Avoids dividing the wild type MIC distribution and isolates with vanA or vanB will be reported resistant.

New vancomycin breakpoints for Enterococcus spp.

Strains with MIC values above 2 mg/L are rare or not yet reported.
New vancomycin breakpoints for *Streptococcus* spp.

EUCAST vancomycin breakpoints

EUCAST strategy for topical antimicrobial agents

- Local concentrations may be high but there are rarely any data correlating MICs to clinical outcome
- There is often anecdotal evidence that an agent is effective in specific conditions in which case it can at least be assumed that wild type isolates are susceptible
- If there is already a clinical breakpoint for the agent, this can be applied for topical preparations also
- Otherwise the ECOFF can serve in lieu of a clinical breakpoint
Brown - Rationale for new or revised EUCAST clinical breakpoints

Topical agents - Retapamulin

• New agent for impetigo and superficial skin infections
• ECOFF set by EUCAST as part of the marketing authorisation by EMA
**Topical agents - Mupirocin**

- Mainly used for nasal decolonization of *Staphylococcus aureus*, particularly MRSA, as part of decolonization regimens.

- May also be used for topical treatment of uncomplicated skin infections such as impetigo, although prolonged use is associated with selection of resistance.

- EUCAST breakpoints are related specifically to nasal decolonization of *S. aureus*.

**Mupirocin clinical studies**

- Topical mupirocin is effective in nasal decolonization of wild type *S. aureus* (MIC ≤1 mg/L)

- Low-level resistant isolates (MIC 8-256 mg/L) are initially cleared as effectively as wild type isolates but recolonization is very common

- Clearance rates for high-level resistant isolates (MIC >256 mg/L) are low

- There is no evidence on outcome for isolates with MICs above the wild type but without a recognised resistance mechanism (MIC ≤4 mg/L)
Other topical agents

- Systemic breakpoints available
  - Chloramphenicol
  - Tetracycline
  - Gentamicin
  - Fusidic acid
  - Polymyxin B
  - Ofloxacin

- ECOFFs to be defined
  - Neomycin (Framycetin)
  - Bacitracin

EUCAST breakpoints under development for less commonly tested organisms

- Clostridium difficile
- Helicobacter pylori
- Listeria monocytogenes
- Pasteurella multocida
- Campylobacter jejuni/coli
- Corynebacterium spp
- Burkholderia cepacia
**Clostridium difficile "breakpoints"**

- Set in consultation with the ESCMID *C. difficile* study group (ESGCD)
- There are no clinical or pharmacodynamic data. Breakpoints are therefore ECOFFs
- Several agents are included for epidemiological purposes only. They are inappropriate for treatment. ECOFFs have been defined when sufficient MIC distributions are available
- Few MIC distributions are available for some agents, so some ECOFFs are tentative

**Clostridium difficile – ECOFFs**

<table>
<thead>
<tr>
<th>Agent</th>
<th>ECOFF (mg/L)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>≤2</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>≤2</td>
<td></td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>≤2</td>
<td></td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>≤4</td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>≤0.004</td>
<td></td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≤0.25</td>
<td>Tested for epidemiological purposes only.</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>≤4</td>
<td></td>
</tr>
</tbody>
</table>

**Helicobacter pylori breakpoints**

- Different test methods may give different results
- There is a shortage of clinical data linking *in vitro* susceptibility to outcome
- All agents are used in combination therapy so outcome data for individual agents are difficult to assess.
**Brown - Rationale for new or revised EUCAST clinical breakpoints**

### Helicobacter pylori proposed breakpoints

<table>
<thead>
<tr>
<th>Agent</th>
<th>S (mg/L)</th>
<th>R (mg/L)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>≤0.12</td>
<td>&gt;0.12</td>
<td>Based on ECOFF. Isolates with higher MICs uncommon. No outcome evidence for MIC &gt;0.12 mg/L.</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≤0.25</td>
<td>&gt;0.5</td>
<td>Clinically validated. Isolates with MIC above 0.5 mg/L have 23S RNA mutation. ECOFF 0.25 mg/L.</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≤1</td>
<td>&gt;1</td>
<td>Largely correlate with gyrA mutations, no outcome data. ECOFF 0.5 mg/L.</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤1</td>
<td>&gt;1</td>
<td>Correspond with mutation in 16S RNA. Resistance rare and no clinical validation. ECOFF 0.25 mg/L.</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>≤1</td>
<td>&gt;1</td>
<td>Correspond with rpoB mutation. Resistance rare and no clinical validation. Few MIC data.</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>≤8</td>
<td>&gt;8</td>
<td>Current, widely accepted breakpoint, but no clinical validation. ECOFF 4 mg/L.</td>
</tr>
</tbody>
</table>

### Subcommittee on antifungal susceptibility testing (EUCAST-AFST)

- **Chair:** J-L Rodriguez-Tudela (ES), Scientific secretary: JP Donnelly (N)
- **AFST Steering Committee members:** MC Arendrup (DK), C Lassl-Flörl (A), W Hope (UK)
- **National representatives:** See EUCAST website [http://www.eucast.org/organization/subcommittees/eucast_afst](http://www.eucast.org/organization/subcommittees/eucast_afst)

### AFST published breakpoints

<table>
<thead>
<tr>
<th>Agent</th>
<th>Organisms</th>
<th>S ≤ (mg/L)</th>
<th>R &gt; (mg/L)</th>
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</thead>
<tbody>
<tr>
<td>Fluconazole</td>
<td>Candida albicans</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Candida glabrata</td>
<td>IE</td>
<td>IE</td>
</tr>
<tr>
<td></td>
<td>Candida tropicalis</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Candida krusei</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Candida parapsilosis</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Candida albicans</td>
<td>0.125</td>
<td>0.125</td>
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<tr>
<td></td>
<td>Candida glabrata</td>
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<tr>
<td></td>
<td>Candida tropicalis</td>
<td>0.125</td>
<td>0.125</td>
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<tr>
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<td>Candida krusei</td>
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<tr>
<td></td>
<td>Candida parapsilosis</td>
<td>0.125</td>
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</table>
AFST proposed breakpoints

<table>
<thead>
<tr>
<th>Agent</th>
<th>Organisms</th>
<th>≤ (mg/L)</th>
<th>&gt; (mg/L)</th>
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<tbody>
<tr>
<td>Amphotericin B</td>
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<tr>
<td>Candida albicans</td>
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<td>1</td>
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<tr>
<td>Candida tropicalis</td>
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<tr>
<td>Candida krusei</td>
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</tr>
<tr>
<td>Candida parapsilosis</td>
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<td>Candida other species</td>
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<tr>
<td>Candida krusei</td>
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<tr>
<td>Candida parapsilosis</td>
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<tr>
<td>Candida guilliermondii</td>
<td>IE</td>
<td>IE</td>
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</table>

Summary
Rationale for EUCAST breakpoints

- EUCAST breakpoints set to harmonize old breakpoints, establish new breakpoints, revise breakpoints
- The background data and rationale for breakpoints are described in EUCAST rationale documents
- The rationale document series will be extended as new breakpoints are set or revised as existing breakpoints are reviewed
Antimicrobial susceptibility testing (AST)

**MIC** (mg/L)

and / or

inhibition zones (mm)

- Clinical categorization (S, I, R)
  - Based on clinical breakpoints
- Interpretive reading
  - Based on resistance mechanisms knowledge
- Application of expert rules
  - Based both on clinical evidence and resistance mechanisms knowledge
Rafael Canton - What is new in EUCAST expert rules?

Interpretive reading of the antibiogram

1. To establish the susceptibility phenotype
2. To infer the potential resistance mechanism
3. To predict previously defined phenotype from the resistance mechanisms

Antibiogram interpretative reading

Importance of bacterial identification

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (mg/L)</th>
<th>Organisms</th>
<th>Potential phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;64</td>
<td>E. coli</td>
<td>AmpC hyperproduction</td>
</tr>
<tr>
<td>Amoxiclav</td>
<td>&gt;32/16</td>
<td>E. coli</td>
<td>plasmid AmpC ESBL + porin deficiency</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>&gt;64</td>
<td>E. coli</td>
<td>ESBL + porin deficiency</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>32</td>
<td>K. pneumonia</td>
<td>ESBL + porin deficiency</td>
</tr>
<tr>
<td>Piper/Tazo</td>
<td>16/4</td>
<td>E. coli</td>
<td>ESBL + porin deficiency</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>&gt;64</td>
<td>K. pneumonia</td>
<td>ESBL + porin deficiency</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>4</td>
<td>E. cloacae</td>
<td>ESBL</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>8</td>
<td>E. cloacae</td>
<td>ESBL</td>
</tr>
<tr>
<td>Cefepime</td>
<td>1</td>
<td>E. cloacae</td>
<td>ESBL</td>
</tr>
</tbody>
</table>

Antibiogram interpretative reading: the classical example

Escherichia coli and ESBL

Courvalin P. ASM News, 1992
Rafael Canton - What is new in EUCAST expert rules?

**Proteus vulgaris**

*hiperproduction of chromosomal b-lactamase (Class A)*

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;256</td>
<td>R</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>8-256</td>
<td>R</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>8-256</td>
<td>S/R</td>
</tr>
<tr>
<td>Piper/Tazo</td>
<td>8-32</td>
<td>S/R</td>
</tr>
<tr>
<td>Amoxiclav</td>
<td>4-8</td>
<td>S/I</td>
</tr>
<tr>
<td>Cefalotin</td>
<td>&gt;256</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>2.4</td>
<td>S</td>
</tr>
<tr>
<td>Cefuroxim</td>
<td>256</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>2.8</td>
<td>R</td>
</tr>
<tr>
<td>Ceftazidim</td>
<td>0.12-0.5</td>
<td>S</td>
</tr>
<tr>
<td>Cefepim</td>
<td>0.5-2</td>
<td>S/I</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5-2</td>
<td>S</td>
</tr>
</tbody>
</table>

**Antimicrobial susceptibility testing (AST)**

- Clinical categorization (S, I, R)
  - Based on clinical breakpoints
- Interpretive reading
  - Based on resistance mechanisms knowledge
- Application of expert rules
  - Based both on clinical evidence and resistance mechanisms knowledge
An expert rule ...

- Description of action to be taken, based on current clinical and/or microbiological evidence, in response to specific antimicrobial susceptibility test results
- Assist clinical microbiologists in the interpretation of antimicrobial susceptibility test results
- Contribute to quality assurance by highlighting anomalous or unlikely results

EUCAST expert rules v2

- Version-2, approved in Vienna, Steering Committee, Feb-2011
- Maintain version-1 structure but rules were revised according with:
  - new clinical and microbiological evidences
  - new defined breakpoints (3rd gen. cephalosporins, ...)
  - current breakpoints tables to avoid potential inconsistencies
    - withdrawal of expert rules of antibiotics for which breakpoints have not been defined. If mention was needed, the ECOFF value was used, but not the clinical category (S or R)
- Nomenclature of microorganisms has been updated

EUCAST expert rules v2

- All expert rules were
  - renumbered and reworded (when needed)
  - edited as: "IF ... THEN ...
- Layout were modified:
  - "agents tested" and "agents affected"
  - "exceptions, scientific basis and comments" (same column)
- Extensive list of performed modifications at the end of the document with a short explanation when needed
- References were added or deleted according with previous modifications
The EUCAST expert rules in antimicrobial susceptibility testing are divided into:

- intrinsic resistances
- exceptional phenotypes
- interpretive rules

**Intrinsic resistance**

- Opposed to acquired or mutational resistance
- Characteristic of all (or almost all) representatives of the species
- The antimicrobial activity of the drug is clinically insufficient or antimicrobial resistance innate as to render it clinically useless
- Antimicrobial susceptibility testing is normally unnecessary
- In these species, “susceptible” results should be viewed with caution (this indicates an error in identification or susceptibility testing)
  - if S is confirmed the drug should preferably not be used
  - R may be expressed at a low level (MIC close to the S breakpoint) although the antibiotic is not considered clinically active
### EUCAST expert rules v2

**Intrinsic resistances affecting Enterobacteriaceae**

<table>
<thead>
<tr>
<th>Rule</th>
<th>Organisms</th>
<th>Ampicillin</th>
<th>Tetracycline</th>
<th>Erythromycin</th>
<th>Cefotaxim</th>
<th>Ceftazidim</th>
<th>Colistin</th>
<th>Amikacin</th>
<th>Gentamicin</th>
<th>Kanamycin</th>
<th>Spectinomycin</th>
<th>Tobramycin</th>
<th>Netilmicin</th>
<th>Nalidixic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Chro. boenzi</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>1.2</td>
<td>Chro. freundt</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>1.3</td>
<td>Enterobacter cloacae</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>1.4</td>
<td>Enterobacter aerogenes</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>1.5</td>
<td>Citrobacter homini</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>1.6</td>
<td>Hafnia alvei</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>1.7</td>
<td>Proteus vulgaris</td>
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<td>R</td>
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<td>R</td>
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<td>R</td>
<td>R</td>
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<tr>
<td>1.8</td>
<td>Morganella morgani</td>
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<td>R</td>
<td>R</td>
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<tr>
<td>1.9</td>
<td>Proteus mirabilis</td>
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<td>1.10</td>
<td>Proteus vigens</td>
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<tr>
<td>1.11</td>
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<tr>
<td>1.13</td>
<td>Providencia retgeri</td>
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<td>R</td>
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<td>R</td>
</tr>
<tr>
<td>1.14</td>
<td>Providencia retgeri</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>1.15</td>
<td>Providencia retgeri</td>
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<td>R</td>
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<td>R</td>
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<td>R</td>
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<td>R</td>
</tr>
<tr>
<td>1.16</td>
<td>Providencia retgeri</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

*P. retgeri* (but not *P. stuartii*) produces a chromosomal AAC(2')-Ia enzyme and should be considered resistant to all aminoglycosides except amikacin and streptomycin.

Some isolates express the enzyme poorly and can appear susceptible to netilmicin in vitro, but should be reported as resistant as mutation can result in overproduction of this enzyme.
Exceptional phenotypes

- Resistances of some bacterial species to particular antimicrobial agents which have not yet been reported or are very rare
- They should be checked as they may also indicate an error in identification or susceptibility testing. If they are confirmed locally:
  - the isolate should be further studied
  - sent to a reference laboratory for independent confirmation
- The may change with time as resistance may develop and increase over time
- There may also be local or national differences. Very rare in one hospital, area or country, may be more common in another

Ertapenem was removed from rule 5.1. … decrease susceptibility to this drug has been reported in isolates with both AmpC-hyperproduction and absence or decreased expression of porins

A. There is clinical evidence that reporting the test result as susceptible leads to clinical failures
B. Evidence is weak and based only on a few case reports or on experimental models. It is presumed that reporting the test result as susceptible may lead to clinical failures
C. There is no clinical evidence, but microbiological data suggest that clinical use of the agent should be discouraged
Evidences

A. There is clinical evidence that reporting the test result as susceptible leads to clinical failures.

B. Evidence is weak and based only on a few case reports or on experimental models. It is presumed that reporting the test result as susceptible may lead to clinical failures.

C. There is no clinical evidence, but microbiological data suggest that clinical use of the agent should be discouraged.
Some major modifications

- **Old 9.1 and 9.8 expert rules** have been deleted
  - ESBL detection and clinical category modification in extended spectrum cephalosporins no longer exist (report as found)
  - carbapenemase detection and clinical category modification in carbapenems no longer exist (report as found)

- **Haemophilus and β-lactams expert rules** have been reworded

- **Old 13.7 expert rule (now 13.6)**
  - nalidixic acid disk diffusion screen test for *Salmonella* and clinical failure of fluoroquinolones due to acquisition of at least one target mutation in gyrA has been substituted for ciprofloxacin MIC criteria of >0.06 mg/L

3rd & 4th gen. cephalosporin breakpoints in Enterobacteriaceae

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>S1</td>
<td>≥4</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>S1</td>
<td>≥4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>S4</td>
<td>≥16</td>
</tr>
<tr>
<td>Cefepime</td>
<td>S8</td>
<td>≥32</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>S4</td>
<td>≥16</td>
</tr>
</tbody>
</table>

*Remember!*

... R category is “≥” in CLSI while “>” in EUCAST

Cefotaxime / Escherichia coli

Antimicrobial wild type distributions of microorganisms – reference database

EUC-NET

<table>
<thead>
<tr>
<th>MIC</th>
<th>5000 observations</th>
<th>32 data subsets</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/L-breakpoints</td>
<td>CLSI</td>
<td>0.06 mg/L</td>
</tr>
<tr>
<td>I/R-breakpoints</td>
<td>EUCAST</td>
<td>0.06 mg/L</td>
</tr>
</tbody>
</table>

Rafael Canton - What is new in EUCAST expert rules?
Rafael Canton - What is new in EUCAST expert rules?

### EUCAST expert rules

**Cephalosporins-ESBLs: Old 9.1 expert rule**

<table>
<thead>
<tr>
<th>Rule No.</th>
<th>Rule Exceptions</th>
<th>Scientific Basis</th>
<th>Grade</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.1</td>
<td>R of 1st or 2nd or 4th gen. cephalosporin (i.e. cefotaxime, ceftaxime, cefpirome, ceftazidime or ceftriaxone) or aztreonam, test for ESBL. If positive, edit the S result for any of these cephalosporins, including 4th gen. agents and for aztreonam, I and edit the I result to R.</td>
<td>A few ESBL producers may appear susceptible to penicillin/β-lactamase inhibitor combinations. The use of these combinations against ESBL-producing isolates with MICs lower than 2 mg/L remains to be fully documented.</td>
<td>C</td>
<td>Wong, Beringer A et al., 2002. Paterson D et al., 2005. Paterson DL et al. 2004. Paterson DL et al., 2002. Bhavnani SM et al. 2006.</td>
</tr>
</tbody>
</table>

* Enterobacteriaceae (for *Klebsiella oxytoca* and *Citrobacter koseri* see 9.3)  
A. There is clinical evidence that reporting the test result as susceptible leads to clinical failures  
B. Evidence is weak based only on a few case reports or on experimental models. It is presumed that reporting the test as susceptible may lead to clinical failures  
C. There is currently no clinical evidence, but microbiological data suggest that clinical use of the agent should be discouraged.

---

**Cefotaxime / Escherichia coli**

Antimicrobial wild type distributions of microorganisms - reference database EUCAST

## 2009

**S/I-breakpoints**  
**I/R-breakpoints**

---

**Cefotaxime / Escherichia coli**

Antimicrobial wild type distributions of microorganisms - reference database EUCAST

## 2010

**S/I-breakpoints**  
**I/R-breakpoints**

---

*CLSI: no ESBL-detection for SIR categorization*  
*EUCAST: no ESBL-detection for SIR categorization*
Cefalosporins

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg/L)</th>
<th>CLSI 2009</th>
<th>EUCAST 2009</th>
<th>EUCAST 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefazolin</td>
<td>&gt;32</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;16</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;32</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefepime</td>
<td>2</td>
<td>S</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>4</td>
<td>S</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Cefepime</td>
<td>2</td>
<td>S</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>4</td>
<td>S</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.25</td>
<td>S</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.03</td>
<td>S</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>

*CLSI: 2:1 ratio; EUCAST: fixed concentration (2 mg/L); NA: not available

Cephalosporin breakpoints and Enterobacteriaceae

**Escherichia coli** (SMART* data base): 19.991 isolates

*SMART: Study for Monitoring Antimicrobial Resistance Trends, 2002-2009
Rafael Canton - What is new in EUCAST expert rules?

**Cephalosporin breakpoints and Enterobacteriaceae**

**BLEE - Escherichia coli** – (SMART* data base): 2.893 isolates

- **100%**
- **90%**
- **80%**
- **70%**
- **60%**
- **50%**
- **40%**
- **30%**
- **20%**
- **10%**
- **0%**

**EUCAST expert rules**

**Carbapenems: Old 9.8 expert rule**

<table>
<thead>
<tr>
<th>Rule No.</th>
<th>Rule Exceptions</th>
<th>Scientific basis</th>
<th>Grade</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.7*</td>
<td>If production of metallo-β-lactamases is confirmed, report the S as I and the I as R for any β-lactam except aztreonam, which should be reported as found.</td>
<td>Metallo-beta-lactamases can hydrolyse all β-lactams except monobactams</td>
<td>B</td>
<td>B. Walsh et al., 2005</td>
</tr>
</tbody>
</table>

**Carbapenem breakpoints and Enterobacteriaceae**

**Enterobacteriaceae**

<table>
<thead>
<tr>
<th>FDA CLSI (2010)*</th>
<th>EUCAST (EMEA) (2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td><strong>S</strong></td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤4</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤2</td>
</tr>
<tr>
<td>Doripenem</td>
<td>≤0,5</td>
</tr>
</tbody>
</table>

* M100-S20U June 2010 **E. coli & K. pneumoniae

* Report as found regardless of the presence or absence of a carbapenemase

**Breakpoint tables for interpretation of MICs and zone diameters**

* The carbapenem breakpoints for Enterobacteriaceae will affect all clinically important resistance mechanisms including the majority of carbapenemases. Some strains that produce carbapenemases are categorized as susceptible with these breakpoints and should be reported as resistant, e.g., the presence or absence of a carbapenemase does not in itself influence the susceptibility of an organism. Various carbapenemase detection and characterization is accomplished in laboratories for effective clinical work.
Rafael Canton - What is new in EUCAST expert rules?

### EUCAST expert rules v2

#### Haemophilus influenzae and β-lactams

**Rule 10.1 v2 (evidence grade A)**

<table>
<thead>
<tr>
<th>Agents tested</th>
<th>Agents affected</th>
<th>Rule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin or penicillin and β-lactamase inhibitors</td>
<td>Ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, ceftriaxone and cefotaxime</td>
<td>If β-lactamase positive THEN report as resistant to ampicillin, amoxicillin and piperacillin-tazobactam. Resistance to ampicillin by production of β-lactamase may be misinterpreted by the disk diffusion technique. Preservation of β-lactamase should be examined with a chromogenic test.</td>
</tr>
</tbody>
</table>

**Rule 10.2 v2 (evidence grade C)**

<table>
<thead>
<tr>
<th>Agents tested</th>
<th>Agents affected</th>
<th>Rule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin or amoxicillin (and β-lactamase inhibitors)</td>
<td>Ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, ceftriaxone and cefotaxime</td>
<td>If β-lactamase negative but amoxicillin resistant, EUCAST THEN report as resistant to ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, ceftriaxone and cefotaxime. ESIAR isolates have reduced affinity of β-lactamase inhibitors. Although piperacillin and piperacillin-tazobactam appear less affected by the β-lactamase-mediated resistance mechanisms, evidence regarding clinical efficacy is lacking.</td>
</tr>
</tbody>
</table>

**Rule 10.3 v2 (evidence grade C)**

<table>
<thead>
<tr>
<th>Agents tested</th>
<th>Agents affected</th>
<th>Rule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-clavulanate (and β-lactamase inhibitors)</td>
<td>Amoxicillin, amoxicillin-clavulanate, amoxicillin-sulbactam, piperacillin-tazobactam, ceftriaxone and cefotaxime</td>
<td>If β-lactamase positive and amoxicillin-clavulanate resistant (in vitro), EUCAST report as resistant to amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, ceftriaxone and cefotaxime. ESIAR isolates produce β-lactamase and have reduced affinity of β-lactamase inhibitors. Resistance to amoxicillin-clavulanate may be misinterpreted by the disk diffusion technique. Preservation of β-lactamase should be examined with a chromogenic test. Significantly more ceftriaxone-resistant isolates were affected by the ESBL-mediated resistance mechanisms. Evidence regarding clinical efficacy is lacking.</td>
</tr>
</tbody>
</table>
Nalidixic ac. (zone diameter), previously recommended for detection of fluoroquinolone resistance, had been removed in breakpoint tables.
- It does not detect qnr-mediated resistance.
- Low-level resistance in Enterobacteriaceae (exception Salmonella spp) is no longer of major interest since high-level resistance is now common in species.

Fluroquinolones breakpoints and Enterobacteriaceae

EUCAST expert rules v2

Salmonella spp. and fluoroquinolones

- There is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by Salmonella spp. with low-level quinolone resistance (MIC >0.064 mg/L). This mainly to S. typhi but there are also case reports of poor response with other Salmonella species.
Rafael Canton - What is new in EUCAST expert rules?

**Salmonella spp., nalidixic acid and fluoroquinolones**

![Graph showing ECOFF values for Salmonella spp. resistant to nalidixic acid and fluoroquinolones]

**Salmonella spp., nalidixic acid and fluoroquinolones**

![Graph showing ECOFF values for Salmonella spp. resistant to nalidixic acid and fluoroquinolones]

**Expert rules in antimicrobial susceptibility testing**

- Version 2
- EUCAST Expert rules in antimicrobial susceptibility testing
- EUCAST, 2011
MIC (mg/l) and / or inhibition zones (mm)

Do your best... but better with EUCAST