Dealing with antibiotic susceptibility and resistance in the 21st Century.

Antimicrobial Susceptibility Testing - How far are we from international consensus?

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Clinical microbiology for Kronoberg and Blekinge
EUCAST Technical Data Coordinator and
Head of the EUCAST Development Laboratory, Växjö, Sweden
## The development of AST

<table>
<thead>
<tr>
<th>Event</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beijerinck in 1889</td>
<td>Used agar diffusion to study the effect of plant growth hormones on bacterial growth.</td>
</tr>
<tr>
<td>Fleming in 1924</td>
<td>Used a “ditch plate” technique for evaluating antimicrobial qualities of antiseptic solutions and later developed the broth dilution technique with turbidity as an endpoint.</td>
</tr>
<tr>
<td>The WHO commissioned the International Collaborative Study (ICS)</td>
<td>Published in 1971 (Ericsson and Sherris).</td>
</tr>
<tr>
<td>The 1970ies - the formation of national breakpoint committees</td>
<td>(DIN, NCCLS, and others) and national disk diffusion AST systems.</td>
</tr>
<tr>
<td>In 2001 nationalcommittees were convinced to take responsibility</td>
<td>for European harmonisation, finalised through EUCAST in 2008.</td>
</tr>
</tbody>
</table>
Antibiotic Sensitivity Testing
Report of an International Collaborative Study

By
HANS M. ERICSSON and JOHN G. SHERRIS

MUNKSCAARD, COPENHAGEN

Micro Spring Meeting 2017
Gunnar Kahlmeter

Has been determining breakpoints since 1986 on

SRGA, SRGA-M, NordicAST, BSAC WP

on AST, CLSI, EUCAST
Ericsson and Sherris (and WHO) were criticized for recommending rigorous standardisation

- **Balows**, head of CDC 1972, commenting on the ICS approach, Balows deemed it impractical and too demanding. It also implied a *level of standardisation that might result in violation of property rights*: ‘I doubt seriously that commercial concerns would willingly or should even be expected to describe or reveal their procedures for impregnation and drying [of discs]. In the USA this might well be regarded as an infringement of their proprietary procedures...’

- **Garrod**: ”I must explain that although I took some part in the International Collaborative Study I have for several years disagreed with the direction it was leading. “The ICS demands a degree of standardisation of the culture medium and of other features of the test, which I believe to be impractical”.

- **Germany**: A national committee on sensitivity testing had voiced concerns in September 1963 that some of Ericsson approaches were ‘too complicated given conditions in German laboratories; it seems possible to implement simplifications without compromising precision’.

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similar arguments are reiterated throughout the following 50 years!

- “…different breakpoints for different species….??”
- “…are we to speciate gramnegatives in UTI?”
- “…we cannot put our recommendations on the internet (1996) – only few laboratories will have access…”
- “…no one will distinguish between E. faecalis and E. faecium – recommendations will have to be the same!”
- “…very few laboratories will ever afford a massspec…”
- “…even if we have a massspec clinicians do not want us to speciate CoNS – it is confusing”
- “…laboratories are not staffed to cope with the extra workload of measuring zone diameters…”
- “…we cannot afford daily QC of AST!”
It used to be so simple....

In the beginning there was one table for everything - one zone diameter break-point (and an MIC correlate) to fit all.
NCCLS First Supplement, 1981

- “useful for anything that would grow”

**TABLE 2. Zone Diameter Interpretive Standards and Approximate Minimum Inhibitory Concentration (MIC) Correlates**

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Disc Content</th>
<th>Resistant</th>
<th>Zone Diameter, nearest whole mm</th>
<th>Approximate MIC Correlates†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intermediate†</td>
<td>Resistant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30 µg</td>
<td>≤ 14</td>
<td>15–16</td>
<td>≥ 32 µg/mL</td>
</tr>
<tr>
<td>Ampicillin§ when testing gram-negative</td>
<td>10 µg</td>
<td>≤ 11</td>
<td>12–13</td>
<td>≥ 32 µg/mL</td>
</tr>
<tr>
<td>enteric organisms and enterococci</td>
<td></td>
<td></td>
<td></td>
<td>≤ 8 µg/mL</td>
</tr>
<tr>
<td>Ampicillin§ when testing staphylococci†</td>
<td>10 µg</td>
<td>≤ 20</td>
<td>21–28</td>
<td>≥ 0.25 µg/mL</td>
</tr>
<tr>
<td>and penicillin G-susceptible microorganisms</td>
<td></td>
<td></td>
<td></td>
<td>β-lactamase§</td>
</tr>
<tr>
<td>Ampicillin§ when testing Haemophilus species‡</td>
<td>10 µg</td>
<td>≤ 19</td>
<td>—</td>
<td>≥ 4 µg/mL</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>10 units</td>
<td>≤ 8</td>
<td>9–12</td>
<td>≤ 16 µg/mL</td>
</tr>
<tr>
<td>Carbencilin when testing the E. coli</td>
<td>100 µg</td>
<td>≤ 17</td>
<td>18–22</td>
<td>≤ 16 µg/mL</td>
</tr>
<tr>
<td>Carbencilin when testing Pseudomonas aeruginosa</td>
<td>100 µg</td>
<td>≤ 13</td>
<td>14–16</td>
<td>≤ 256 µg/mL</td>
</tr>
<tr>
<td>Cefamandole§</td>
<td>30 µg</td>
<td>≤ 14</td>
<td>15–17</td>
<td>≤ 128 µg/mL</td>
</tr>
<tr>
<td>Cefotaxime§</td>
<td>30 µg</td>
<td>≤ 14</td>
<td>15–22†</td>
<td>≤ 32 µg/mL</td>
</tr>
<tr>
<td>Cefoxitin§</td>
<td>30 µg</td>
<td>≤ 14</td>
<td>15–17</td>
<td>≤ 8 µg/mL</td>
</tr>
<tr>
<td>Cephalexin§</td>
<td>30 µg</td>
<td>≤ 14</td>
<td>15–17</td>
<td>≤ 8 µg/mL</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 µg</td>
<td>≤ 12</td>
<td>13–17</td>
<td>≤ 25 µg/mL</td>
</tr>
<tr>
<td>Clindamycin§</td>
<td>2 µg</td>
<td>≤ 14</td>
<td>15–16</td>
<td>≤ 12.5 µg/mL</td>
</tr>
<tr>
<td>Colistin</td>
<td>10 µg</td>
<td>≤ 8</td>
<td>9–10</td>
<td>≤ 2 µg/mL</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10 µg</td>
<td>≤ 13</td>
<td>14–17</td>
<td>≤ 1 µg/mL</td>
</tr>
<tr>
<td>Gentamicin§</td>
<td>10 µg</td>
<td>≤ 12</td>
<td>13–14</td>
<td>≤ 1 µg/mL</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 µg</td>
<td>≤ 13</td>
<td>14–17</td>
<td>≤ 1 µg/mL</td>
</tr>
<tr>
<td>Methicillin</td>
<td>5 µg</td>
<td>≤ 9</td>
<td>10–13</td>
<td>≤ 1 µg/mL</td>
</tr>
<tr>
<td>Nafcillin§</td>
<td>1 µg</td>
<td>≤ 10</td>
<td>11–12</td>
<td>≤ 1 µg/mL</td>
</tr>
<tr>
<td>Nalidixic Acid§</td>
<td>30 µg</td>
<td>≤ 13</td>
<td>14–18</td>
<td>≤ 2 µg/mL</td>
</tr>
<tr>
<td>Neomycin</td>
<td>30 µg</td>
<td>≤ 12</td>
<td>13–16</td>
<td>≤ 4 µg/mL</td>
</tr>
<tr>
<td>Nitrofurantoin§</td>
<td>300 µg</td>
<td>≤ 14</td>
<td>15–16</td>
<td>≤ 100 µg/mL</td>
</tr>
<tr>
<td>Oxacillin§</td>
<td>1 µg</td>
<td>≤ 10</td>
<td>11–12</td>
<td>≤ 25 µg/mL</td>
</tr>
<tr>
<td>Penicillin G when testing staphylococci§</td>
<td>10 units</td>
<td>≤ 20</td>
<td>21–28</td>
<td>≤ 2 µg/mL</td>
</tr>
<tr>
<td>Penicillin G when testing other microorganisms</td>
<td>10 units</td>
<td>≤ 14</td>
<td>15–16</td>
<td>≤ 0.1 µg/mL</td>
</tr>
</tbody>
</table>

† Approximate MIC Correlates:
- Resistant: ≥ concentration
- Susceptible: ≤ concentration
- Intermediate: ≥ concentration

§ β-lactamase

‡ β-lactamase

§ β-lactamase

‡ β-lactamase
SRGA (RAF) had a similar table

All breakpoints were valid for all species and the correlation/regression between MIC and inhibition zone diameters was a general, multiple species regression.
### Breakpoint committees in the old days!

<table>
<thead>
<tr>
<th>Committee</th>
<th>Country</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIN (G Linzenmeier)</td>
<td>Germany</td>
<td>1973?</td>
</tr>
<tr>
<td>NCCLS (later CLSI) (A Barry)</td>
<td>USA</td>
<td>1975</td>
</tr>
<tr>
<td>NWGA (K Mellby)</td>
<td>Norway</td>
<td>1978</td>
</tr>
<tr>
<td>SRGA (RAF) (LO Kallings)</td>
<td>Sweden</td>
<td>1979</td>
</tr>
<tr>
<td>CA-SFM (Y Chabbert)</td>
<td>France</td>
<td>1980</td>
</tr>
<tr>
<td>WRG (later CRG) (P Mouton)</td>
<td>Netherlands</td>
<td>1981</td>
</tr>
<tr>
<td>BSAC WP on AST (I Phillips)</td>
<td>The UK</td>
<td>1988</td>
</tr>
<tr>
<td>Committee</td>
<td>Amoxicillin</td>
<td>Cefotaxime</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
<td>------------</td>
</tr>
<tr>
<td>BSAC (UK)</td>
<td>8 / 16</td>
<td>2 / 2</td>
</tr>
<tr>
<td>CA-SFM (F)</td>
<td>4 / 16</td>
<td>4 / 32</td>
</tr>
<tr>
<td>CRG (NL)</td>
<td>2 / 16</td>
<td>4 / 8</td>
</tr>
<tr>
<td>DIN (D)</td>
<td>2 / 8</td>
<td>2 / 8</td>
</tr>
<tr>
<td>NCCLS (USA)</td>
<td>8 / 16</td>
<td>8 / 32</td>
</tr>
<tr>
<td>NWGA (N)</td>
<td>0.5 / 8</td>
<td>1 / 2</td>
</tr>
<tr>
<td>SRGA (S)</td>
<td>1 / 8</td>
<td>0.5 / 1</td>
</tr>
</tbody>
</table>

All of us managed to come up with different breakpoints.

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Breakpoints can fail in several ways!

- **Fail to predict failure (undercall resistance)**
  - CLSI breakpoints for 3rd generation cephalosporins (1980 – 2001)

- **Fail to predict success (overcall resistance)**
  - Penicillin breakpoints in *S. pneumoniae* in pneumonia

- **Generally fail to be useful (lack of correlation with either success or failure)**
  - Erythromycin breakpoints in *H. influenzae* (dividing a WT population in three SIR-categories)
The breakpoint committees did not agree...

• ...not because we disagreed
• ...but we were out of sync
• ...and did not communicate with each other
• ...and we all knew best
EUCAST was formed by ESCMID in 1997 and restructured in 2001.....

I was asked to chair EUCAST and realised that Ian Phillips’ mistake was to have ignored the national committees.

Within 12 months, all national committees agreed to take joint responsibility for harmonising European breakpoints.

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EUCAST General Committee
All European Countries + many countries outside Europe
EUCAST Steering Committee

Subcommittees
Antifungals
Anaerobes
Expert Rules
Detection of resistance mechanisms
The relationship between phenotypic susceptibility testing and WGS
MIC distributions and ECOFFs

National Breakpoint Committees
D, F, N, NL, S, UK

Expert groups
EUCAST leadership

Chair
• Ian Philips (UK) 1997 – 2001
• Gunnar Kahlmeter (S) 2001 – 2012
• Rafael Canton (ES) 2012 – 2016
• Christian Giske (S) 2016 –

Scientific secretary
• Derek Brown (UK) 1997 – 2016
• John Turnidge (AUS) 2017 –
EUCAST Subcommittees

- AFST - Antifungal susceptibility testing
- Anaerobes
- Mycobacteria
- Intrinsic resistance and expert rules
- Detection of resistance mechanisms of clinical or public health importance
- Relationship between WGS and Phenotypic AST
- MIC distributions and the setting of ECOFFs
The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee.

Review article


Authors

Ellington MJ¹, Ekelund O², Aarestrup FM³, Canton R⁴, Doumith M¹, Giske C⁵, Grundman H⁶, Hasman H⁷, Holden MT⁸, Hopkins KL¹, Iredell J⁹, Kahlmeter G², Köser CU¹⁰, MacGowan A¹¹, Mevius D¹², Mulvey M¹³, Naas T¹⁴, Peto T¹⁵, Rolain JM¹⁶, Samuelsen Ø¹⁷, Woodford N¹⁸.
The EUCAST decision process

Anyone can suggest......

Major decisions for open consultation (all breakpoint decisions)
EUCAST Consultations

Current consultations

- Consultation - letter of invitation 3 March, 2017 - 14 April, 2017: Revision of “EUCAST guidelines for detection of resistance mechanisms and specific resistances of Clinical and/or epidemiological importance”. Form to be used for comments (no later than 14 April, 2017)

- Consultation - letter of invitation 9 March, 2017 - 14 May, 2017: “EUCAST discussion document (v 3) on MIC distributions and the determination of epidemiological cut-off values (ECOFFs)” - from the EUCAST Subcommittee on MIC distributions and ECOFFs. Form to be used for comments (no later than 14 May, 2017)

Consultations with comments and responses:

- Proposed breakpoints for Aerococcus spp and Kingella kingae - comments and responses.

- Proposed revision of fluoroquinolone breakpoints. - Comments and responses.

- Proposed revision of the colistin breakpoint for Pseudomonas aeruginosa. - Comments and responses.

- Report from the EUCAST Subcommittee on the role of whole genome sequencing (WGS) in antimicrobial susceptibility testing. - Comments and responses.

- Wide consultation the EUCAST proposed changes in the definition of the intermediate category. - Comments and responses.

- Nitrofurin breakpoints - Comments and responses.

- The Intermediate category - the need for a modified definition. Document, comments and responses Sept 2016
The first consultation will be followed by a second consultation 2017.


Comments not entered into the designated document (Document for comments) will not be considered.
Implementation of EUCAST breakpoints, April 2017

% Laboratories
- >50%
- 10-50%
- <10%
- No information

Countries not on this map: Australia, Brazil, Canada, Iceland, Israel, Morocco, New Zealand, South Africa, USA.
AST guidelines used in UK NEQAS External Quality Assurance
(630-750 participants per year from a total of 40 countries)
EUCAST NACs
NAC – National AST Committee

- Implementation of EUCAST on a national level
- Translation of guidelines
- National website
- Education of health care professionals
- Liaison with EUCAST (general committee, steering committee and consultations)
National AST Committees (NACs), April 2017

- **Yes**
- **In the process of forming a NAC**
- **No**
- **No information**

**Other countries:** Australia, Brazil, China, Canada, Iceland, Israel, Morocco, New Zealand, South Africa, USA
Countries with a NAC operating under EUCAST standards

Countries with interest to establish a NAC under EUCAST standards
>50 000 hits per month

www.eucast.org
What is new in EUCAST 2016/17?

• New organisms – breakpoints 2016/17
  – Aerococcus spp, Kingella kingae (Aeromonas, Plesiomonas).
• Review of breakpoints
  – Revised: Colistin, fluoroquinolones – finalised
• Disk diffusion methods for existing agents
  – Nitroxoline, fosfomycin, methicillin resistance in Coag,neg staphylococci.
  – Aerococcus spp, Kingella kingae, (Anaerobes)
• Detection of methicillin resistance in CoN Staphylococci
• The relationship between WGS and phenotypic AST (2016)
• What to do when there are no breakpoints? (SOP 2016)
• Instruction videos (commissioned by WHO) 5 + 5
• Methods for the detection of resistance mechanisms of clinical and/or public health importance (revision).
What is next?

• Reviewing breakpoints for carbapenems, tigecycline and aminoglycosides.
• Reviewing breakpoints in national and international endocarditis recommendations.
• Revising Intrinsic resistance and Expert Rules.
• Revising Methods for detecting resistance mechanisms.
• Revising the definition of INTERMEDIATE.
• Rapid direct AST from blood culture bottles – standardised method and breakpoints for 4, 6 and 8-hour reading.
• Warnings against non-functioning commercial products.

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Warnings by EUCAST
EUCAST warnings concerning antimicrobial susceptibility testing products or procedures.

The EUCAST disk diffusion development laboratories, a network of laboratories coordinated from the EUCAST development laboratory in Växjö, Sweden, from time to time discover products (disks, media batches, gradient tests or procedures) which are not performing to the expected standard. When this is the case we inform the manufacturer and publish a warning on this page.

We do not systematically test all products so the lack of a warning does not imply that there is no problem with the product in question.

Laboratories which experience problems with a susceptibility test method, and suspect that this may be related to a particular product, may contact EUCAST for advice.

1. Problems with piperacillin/tazobactam gradient tests from two manufacturers (see below).
2. Wide variation in disk quality in 16 disks from nine manufacturers (see below)
The EUCAST Development Laboratories evaluates AST material (spontaneously or because of problems detected by user or company).

Disks, media, gradient tests have been investigated.

Warnings are issued on the website.

Currently there are warnings against

- Gradient tests for piperacillin-tazobactam from two manufacturers.
- Disks from several manufacturers.
- Colistin gradient tests from two manufacturers and against colistin disk diffusion testing in general.
Colistin AST study layout and isolates

- 75 Gram-negative organisms with colistin MICs 0.25-128 mg/L
  - 14 *E. coli* (Salmonella not included in this summary)
  - 18 *K. pneumoniae*
  - 21 *P. aeruginosa*
  - 22 *Acinetobacter* spp.
- Colistin BMD on frozen panels (TREK) as reference
- Methods evaluated:
  - 3 BMD methods with freeze-dried antibiotics:
    - SEMPA1: custom Sensititre plate (TREK/Thermo Fisher Scientific)
    - MICRONAUT-S: 96-well colistin plate (MicLev)
    - MIC-Strip: Single strips (12 wells) for BMD (MicLev)
  - Gradient tests:
    - Etest: Oxoid MH, BBL MH and MHE (bioMerieux recommended media)
    - MIC Test Strip (MTS): Oxoid and BBL MH
## Summary of results (frozen MICs as reference)

### New breakpoints for Pseudomonas (2/2) = 2/2 for all organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>E. coli and K. pneumoniae (n=32)</th>
<th>P. aeruginosa (n=21)</th>
<th>Acinetobacter spp. (n=22)</th>
<th>All isolates (n=75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL MIC range (mg/L)</td>
<td>0.25-32</td>
<td>0.25-128</td>
<td>0.5-32</td>
<td>0.25-128</td>
</tr>
<tr>
<td>SEMPA1*</td>
<td>27</td>
<td>19</td>
<td>20</td>
<td>66 (96%)</td>
</tr>
<tr>
<td>MICRONAUT-S</td>
<td>31</td>
<td>21</td>
<td>20</td>
<td>72 (96%)</td>
</tr>
<tr>
<td>MIC-Strip</td>
<td>31</td>
<td>21</td>
<td>22</td>
<td>74 (99%)</td>
</tr>
<tr>
<td>Etest/Oxoid MH</td>
<td>27</td>
<td>13</td>
<td>13</td>
<td>53 (71%)</td>
</tr>
<tr>
<td>Etest/BBL MH</td>
<td>20</td>
<td>11</td>
<td>1</td>
<td>32 (43%)</td>
</tr>
<tr>
<td>Etest/MHE</td>
<td>24</td>
<td>9</td>
<td>2</td>
<td>35 (47%)</td>
</tr>
<tr>
<td>MTS/Oxoid MH</td>
<td>19</td>
<td>12</td>
<td>9</td>
<td>40 (53%)</td>
</tr>
<tr>
<td>MTS/BBL MH</td>
<td>24</td>
<td>10</td>
<td>13</td>
<td>47 (63%)</td>
</tr>
</tbody>
</table>

### EA = essential agreement = MICs within ± 1 dilution of reference (truncated values excluded)

### ME = Major Error (R with test method, S with reference)

### VME = Very Major Error (S with test method, R with reference)

* The total number of tests for calculation of EA was 28 for E. coli and K. pneumoniae and 19 for P. aeruginosa due to truncation of MICs.
# Checking on manufacturers

Jenny Åhman et al, Poster 0824, ECCMID 2016

Table 1. Evaluation of disks from nine manufacturers vs. EUCAST QC targets and ranges**.

1 = First Study, 2 = Follow-up Study

<table>
<thead>
<tr>
<th>Antimicrobial disk</th>
<th>Bio-Rad</th>
<th>Lieofilchem</th>
<th>BD</th>
<th>Abtek</th>
<th>SirScan</th>
<th>Oxoid</th>
<th>HiMedia</th>
<th>Bioanalyse</th>
<th>Mast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Benzylopenicillin 1 unit</td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
<td>H</td>
<td></td>
<td>H</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Amoxicillin-clav. 30 µg</td>
<td>H</td>
<td>H*</td>
<td>F</td>
<td></td>
<td>H</td>
<td></td>
<td>H</td>
<td>NA</td>
<td>L</td>
</tr>
<tr>
<td>Piperacillin-tazo. 36 µg</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td></td>
<td>H</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Oxacillin 1 µg</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Mecillinam 10 µg</td>
<td></td>
<td></td>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime 5 µg</td>
<td>NA</td>
<td></td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime 30 µg</td>
<td>H*</td>
<td>H*</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftazidime 10 µg</td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L*</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>Meropenem 10 µg</td>
<td>H</td>
<td>H*</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin 5 µg</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td></td>
<td>H</td>
<td></td>
<td>H*</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin 10 µg</td>
<td>L</td>
<td>L L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pefloxacin 5 µg</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Gentamicin 10 µg</td>
<td>NA</td>
<td>NA</td>
<td>H</td>
<td></td>
<td>L</td>
<td></td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobramycin 10 µg</td>
<td>NA</td>
<td>NA</td>
<td>H</td>
<td></td>
<td>L</td>
<td></td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin 15 µg</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td></td>
<td>L</td>
<td></td>
<td>H</td>
<td>H</td>
<td>L*</td>
</tr>
<tr>
<td>Tetracycline 30 µg</td>
<td>L</td>
<td>L*</td>
<td>L</td>
<td></td>
<td>L*</td>
<td></td>
<td></td>
<td>H</td>
<td>L</td>
</tr>
</tbody>
</table>

**Data from the first study has been reanalyzed due to changes in QC criteria between 2015 and 2016.

These data, including information on disk lot numbers, are published on www.eucast.org.

- Mean value within ± 1 mm of the target value
- Mean value >1 mm but within ± 2 mm of the target value
- Mean value >2 mm from target value but still within the QC range
- Mean value out of the QC range
- Disk included in first study, but not supplied for follow-up study

NA = Not Available
H = High, mean value > 1 mm above target
L = Low, mean value > 1 mm below target
* One or more readings out of QC range
Redefining 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 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.”
Intermediate – current definition

• uncertain therapeutic effect (pharmacology/microbiology)
• where the drugs are physiologically concentrated (pharmacokinetics)
• when a high dosage of drug can be used (toxicology)
• a buffer zone (methodology)
Definitions of Intermediate

• Some parts point to shortcomings which should be attended to by the laboratory.

• Other parts of the definition encourages the clinician to increase the exposure and this part implies no AST uncertainty.
Interpreting Intermediate

• If the results in the laboratory are in doubt (buffer zone, reading difficulties, trailing endpoints, colonies in zone etc)
  – It is the responsibility of the laboratory to solve this (repeat the test, or choose another test, or advice against the use of the agent by reporting an “R”).

• If circumstances are such that exposure needs to be increased....
  – It is the responsibility of the clinician on a signal from the laboratory (and there should be no doubt about how the signal should be interpreted).
"High dose"

<table>
<thead>
<tr>
<th>Carbapenems</th>
<th>Standard dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doripenem</td>
<td>0.5 g x 3 iv over 1 hour</td>
<td>1 g x 3 iv over 4 hours</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>1 g x 1 iv over 30 minutes</td>
<td>None</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>
</tr>
<tr>
<td>Meropenem</td>
<td>1 g x 3 iv over 30 minutes</td>
<td>2 g x 3 iv over 30 minutes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monobactams</th>
<th>Standard dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam</td>
<td>1 g x 3 iv</td>
<td>2 g x 4 iv</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fluoroquinolones</th>
<th>Standard dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<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>
</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>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.4 g x 1 oral or 0.4 g x 1 iv</td>
<td>None</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>Laboratory test reagent only</td>
<td>Laboratory test reagent only</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>0.4 g x 2 oral</td>
<td>None</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>
</tr>
</tbody>
</table>

... for some betalactams more.
Intermediate (I): A microorganism is categorised as intermediate when there is a high likelihood of therapeutic success because exposure (activity)* is enhanced (1) by adjusting the dosing regimen, or (2) because the antimicrobial agent is concentrated at the site of infection.

*exposure (activity) refers to antimicrobial concentration profiles found in the pharmacokinetic-pharmacodynamic indices that are relevant to the drug class: the area-under-the-drug concentration curve of the unbound drug, divided by the MIC ($f_{AUC}/MIC$ ratio), or the percentage of the dosing interval that the unbound drug is above the MIC ($%fT>MIC$).
AST methods
Methods for susceptibility testing

• **Phenotypic test methods**
  based on *antimicrobial activity (MIC)* and *breakpoints*
  – MIC, disk diffusion, automated systems like Phoenix, Vitek2, Microscan
  – Predict susceptibility and resistance
  – Quantifiable

• **Genotypic test methods**
  based on the detection of a *resistance gene* or its *product*
  – meca, vanA, vanB, ...PBP2, ... betalactamase detection (enzyme detection, Maldi Tof)
  – Predict resistance, not sensitivity
  – Not quantifiable
  – Useful for epidemiological purposes

• **By deduction – “expert rules”**
  – If MRSA then report all betalactam antibiotics R – or soon not?
    If ESBL-positive, then report betalactam antibiotics R – but not any longer!
    If erythromycin-resistant, then report all macrolide antibiotics as R;
  – Some rules predict susceptibility, others resistance.
  – Not quantifiable.
  – Unreliable!
Issues in AST methods

• Daily QC testing mandatory
  – Accreditation authorities being advised

• Recurring issues and development delays in semi-automated AST (Microscan, Phoenix, Vitek2)

• Poor quality of disks from some manufacturers

• Recurring issues with gradient tests

• Colistin – broth micro dilution. EUCAST warns against disk diffusion and gradient tests.
The future - methods

• Rapid WGS with rapid identification of epidemiological markers, resistance genes, virulence genes.
• Rapid phenotypic AST (4 – 8 h) based on MIC determination with rapid methods for detecting “growth”
• Rapid phenotypic AST (4 – 8 h) based on automated disk diffusion with early reading against specific databases and breakpoint tables.
• ....?
The future - breakpoints

• Species specific breakpoints
  – An increasing number of breakpoints will be valid only for defined organisms

• Disease specific breakpoints
  – Meningitis vs. other infections

• Dose specific breakpoints
  – The new definition of INTERMEDIATE

• Individualised breakpoints (disease activity, PK/PD etc)?

• The introduction of a fourth category (LLR and HLR)?
The introduction of a fourth AST category

S, I, LLR, HLR

- **Susceptible** – successful therapy with standard dosages and administration mode.
- **Intermediate** – successful therapy with approved high dose therapy.
- **Low Level Resistance** – therapy when higher than approved dosages might be effective.
  - Daptomycin and Enterococcus spp in endocarditis, 3rd gen cephalosporins and ESBL-producing Enterobacteriaceae, carbapenems in carbapenemase producing Enterobacteriaceae.
- **High Level Resistance** – therapy will fail irrespective of dose.
The future – breakpoint committees

• EUCAST is taking over the scene through evolutionary principles (survival of the fittest and in this case the most cost/effective)

• CLSI and EUCAST will never merge but will continue to harmonise methodology and some breakpoints.

• Recruitment of new SC members through NAC representatives worldwide.

• EUCAST may consider changing its name one day – but so far very few proposals to do so.
Thank you!