EXPERT RULES IN ANTIMICROBIAL SUSCEPTIBILITY TESTING

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Antimicrobial susceptibility testing

- 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
Antimicrobial susceptibility testing

Clinical breakpoints

- The ultimate goal of clinical breakpoints is using MIC values to separate strains where there is a high likelihood of treatment success (S) from those where treatment is more likely to fail (R)

- They are not primarily defined to detect resistant bacteria

- They are mainly derived from prospective human clinical studies comparing outcomes with the MICs of the infecting pathogen and supported with MIC distributions and Pk/Pd studies

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

This has not been always the case in the past!
Antimicrobial susceptibility testing

During more than twenty years interpretive reading of the antibiogram have been used to:

- infer resistance mechanisms behind resistant phenotypes
- identify resistant organisms for infection control purposes
- apply *an expert rule* and modify (when needed!) previous clinical categorization

This approach was partially needed due to inadequate breakpoints!

Courvalin P. ASM News 1992;58:368-75

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

- Interpretative reading: the classical example

ESBL positive isolate

resistant to all cephalosporins and azthreonam (irrespective of MICs)

expert rule
1.- To establish the susceptibility phenotype

2.- To infer the potential resistance mechanism

3.- To predict previously defined phenotype from the resistance mechanisms

Courvalin P, ASM News, 1992
Antibiogram interpretative reading

Importance of bacterial identification

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Amox/clav</td>
<td>&gt;32/16</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>32</td>
</tr>
<tr>
<td>Piper/Tazo</td>
<td>16/4</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>8</td>
</tr>
<tr>
<td>Cefepime</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Potential phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>AmpC hyperproduction plasmid AmpC</td>
</tr>
<tr>
<td></td>
<td>ESBL + porin deficiency</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>ESBL + porin deficiency</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>ESBL</td>
</tr>
</tbody>
</table>
**Proteus vulgaris**

hyperproduction of chromosomal β-lactamase (Class A)

<table>
<thead>
<tr>
<th>MIC</th>
<th>Interp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;256</td>
<td>R</td>
</tr>
<tr>
<td>8-256</td>
<td>R</td>
</tr>
<tr>
<td>8-&gt;256</td>
<td>S/R</td>
</tr>
<tr>
<td>8-32</td>
<td>S/R</td>
</tr>
<tr>
<td>4-8</td>
<td>S/I</td>
</tr>
<tr>
<td>&gt;256</td>
<td>R</td>
</tr>
<tr>
<td>2-4</td>
<td>S</td>
</tr>
<tr>
<td>&gt;256</td>
<td>R</td>
</tr>
<tr>
<td>2-8</td>
<td>R</td>
</tr>
<tr>
<td>0,12-0,5</td>
<td>S</td>
</tr>
<tr>
<td>0,5-2</td>
<td>S/I</td>
</tr>
<tr>
<td>0,5-2</td>
<td>S</td>
</tr>
</tbody>
</table>
EUCAST expert rules: definition

- **Expert rules in antimicrobial susceptibility testing (AST)**
  - describe actions to be taken on the basis of specific AST results
  - based on clinical breakpoints & resistance mechanism knowledge
  - assist clinical microbiologists in the interpretation of AST results
  - contribute to quality assurance by highlighting anomalous results
  - should be in agreement with clinical breakpoints

Winstanley T, Courvalin P. Clin Microbiol Rev 2011; 24: 515–56
http://www.eucast.org
EUCAST Expert rules are a tabulated collection of expert knowledge on intrinsic resistances, exceptional resistance phenotypes and interpretive rules that may be applied to antimicrobial susceptibility testing in order to reduce errors and make appropriate recommendations for reporting particular resistances.

EUCAST Expert rules (version 2.0 available from 29 Oct, 2011)

Archive:
- EUCAST Expert rules (version 1.0 valid until 29 Oct, 2011)
EUCAST expert rules in antimicrobial susceptibility testing

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Clin Microbiol Infect, 2011 Oct 21 [Epub ahead of print]
The EUCAST expert rules in antimicrobial susceptibility testing are divided into:

- intrinsic resistances
- exceptional phenotypes
- interpretive rules

http://www.eucast.org
EUCAST expert rules v2: intrinsic resistance

- Characteristic of all or almost all isolates of the bacterial species
- The antimicrobial activity of the drug is clinically insufficient or antimicrobial resistance is innate, rendering it clinically useless
- Antimicrobial susceptibility is unnecessary
EUCAST expert rules v2: intrinsic resistance
Gram-negative bacteria other than Enterobacteriaceae and non-fermentative Gram-negative bacteria listed are also intrinsically resistant to glycopeptides, lincosamides, daptomycin, and linezolid.

### Table: EUCAST expert rules v2: intrinsic resistance

<table>
<thead>
<tr>
<th>Rule no.</th>
<th>Organisms</th>
<th>Macrolides</th>
<th>Fusidic acid</th>
<th>Streptogramins</th>
<th>Trimethoprim</th>
<th>Nalidixic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td><em>Haemophilus influenzae</em></td>
<td>I</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td><em>Moraxella catarrhalis</em></td>
<td>I</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td><em>Neisseria spp.</em></td>
<td></td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td><em>Campylobacter fetus</em></td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>3.5</td>
<td><em>Campylobacter jejuni, Campylobacter coli</em></td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

R, resistant; I, intermediate.
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.
## EUCAST expert rules v2: exceptional phenotypes

<table>
<thead>
<tr>
<th>Rule no.</th>
<th>Organisms</th>
<th>Exceptional phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Any <em>Enterobacteriaceae</em> (except Proteae)</td>
<td>Resistant to meropenem and/or imipenem&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5.2</td>
<td><em>Serratia marcescens</em> and <em>Proteae</em></td>
<td>Susceptible to colistin</td>
</tr>
<tr>
<td>5.3</td>
<td><em>Pseudomonas aeruginosa</em> and <em>Acinetobacter spp.</em></td>
<td>Resistant to colistin</td>
</tr>
<tr>
<td>5.4</td>
<td><em>Haemophilus influenzae</em></td>
<td>Resistant to any third-generation cephalosporin, carbapenems, and fluoroquinolones</td>
</tr>
<tr>
<td>5.5</td>
<td><em>Moraxella catarrhalis</em></td>
<td>Resistant to ciprofloxacin and any third-generation cephalosporin</td>
</tr>
<tr>
<td>5.6</td>
<td><em>Neisseria meningitidis</em></td>
<td>Resistant to any third-generation cephalosporin and fluoroquinolones</td>
</tr>
<tr>
<td>5.7</td>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Resistant to third-generation cephalosporin and spectinomycin</td>
</tr>
</tbody>
</table>

<sup>a</sup>Except in countries in which carbapenemase-producing *Enterobacteriaceae* are not rare.
## EUCAST expert rules v2

- Actions to be taken on the basis of specific AST results

| Rule no. | Organisms         | Agents tested                                           | Agents affected | Rule                                                                 | Exceptions, scientific basis and comments                                                                 | Evidence grade | References                                      |
|----------|-------------------|---------------------------------------------------------|-----------------|----------------------------------------------------------------------|----------------------------------------------------------------******************************************|---------------|-------------------------------------------------|
| 8.1      | *Staphylococcus* spp. | Oxacillin, cefoxitin (disk diffusion) or detection of mecA gene or PBP2a | All beta-lactams | IF resistant to *isoxazolyl* penicillins (as determined with oxacillin, cefoxitin, or by detection of mecA-gene or PBP2a) THEN report as resistant to all β-lactams. | Production of PBP2a (encoded by mecA) leads to cross resistance to β-lactams except ceftobiprole and ceftaroline. | A             | Chambers HF et al, 1990 Page MG et al, 2006     |
Evidences of expert rules

A. There is good clinical evidence that reporting the test results 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.

http://www.eucast.org
### Evidences

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

<table>
<thead>
<tr>
<th>Rule no.</th>
<th>Organisms</th>
<th>Agents tested</th>
<th>Agents affected</th>
<th>Rule</th>
<th>Exceptions, scientific basis and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1</td>
<td><em>Staphylococcus</em> spp.</td>
<td>Oxacillin, cefoxitin (disk diffusion) or detection of mecA gene or PBP2a</td>
<td>All beta-lactams</td>
<td>IF resistant to isoxazolyl-penicillins (as determined with oxacillin, cefoxitin, or by detection of mecA-gene or of PBP2a) THEN report as resistant to all β-lactams.</td>
<td>Production of PBP2a (encoded by mecA) leads to cross resistance to β-lactams except ceftobiprole and cefiaroline.</td>
</tr>
</tbody>
</table>
## Evidences

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.

<table>
<thead>
<tr>
<th>Rule no.</th>
<th>Organism</th>
<th>Agents tested</th>
<th>Agents affected</th>
<th>Rule</th>
<th>Exceptions, scientific basis and comments</th>
<th>Evidence grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.6</td>
<td><em>Salmonella</em> spp.</td>
<td>Ciprofloxacin</td>
<td>All fluoroquinolones</td>
<td>If ciprofloxacin MIC ≥ 0.06 mg/L, THEN report as resistant to all fluoroquinolones.</td>
<td>Evidence for clinical failure of fluoroquinolones in case of resistance due to the acquisition of at least one target mutation in gyrA.</td>
<td>A (<em>Salmonella typhi</em>) B (other <em>Salmonella</em> spp.)</td>
</tr>
</tbody>
</table>
Evidences

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

<table>
<thead>
<tr>
<th>Rule no.</th>
<th>Organisms</th>
<th>Agent tested</th>
<th>Agents affected</th>
<th>Rule</th>
<th>Exceptions, scientific basis and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.1</td>
<td><em>Staphylococcus</em> spp.</td>
<td>Kanamycin</td>
<td>Amikacin</td>
<td>IF kanamycin MIC &gt; 8 mg/L, THEN report resistant to amikacin.</td>
<td>Resistance to kanamycin is generally due to the production of APH(3′)-3, ANT(4′)(4″)-I or bifunctional APH(2′)-AAC(6) enzymes that determine loss of synergism of kanamycin and amikacin with β-lactams and glycopeptides irrespective of MIC values.</td>
</tr>
</tbody>
</table>
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
### 3<sup>rd</sup> to 4<sup>th</sup> gen. cephalosporin breakpoints in Enterobacteriaceae

<table>
<thead>
<tr>
<th>Cefalosporins</th>
<th>CLSI (2010-12)</th>
<th>EUCAST (2009-12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≤1 (8)*</td>
<td>≥4 (64) =</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤1 (8)</td>
<td>≥4 (64) =</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≤4 (8)</td>
<td>≥16 (32)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>≤8</td>
<td>≥32</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≤4 (8)</td>
<td>≥16 (32)</td>
</tr>
</tbody>
</table>

*2009

**Remember!**

... R category is “≥” in CLSI while “>” in EUCAST
CLSI and EUCAST “new” breakpoints were supported on Pk/Pd data, animal models and clinical outcome data.

Monte-Carlo simulations and target attainment rate (TAR) for intravenous ceftriaxone 2 g every 24 h in Enterobacteriaceae in a murine thigh infection model: Cephalosporin % T>MIC and microbiological efficacy.

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>100</td>
<td>100</td>
<td>97</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>0.5</td>
<td>100</td>
<td>100</td>
<td>72</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>90</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td>99</td>
<td>29</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>4.0</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

MacGowan. CMI 2008; 14(Suppl 1):166-8

Andes & Craig. CMI 2005; 11(Suppl 6):10-7
Probability of target attainment (PTA) for ceftazidime

- 2 log drop in viable Gram-negatives requires 50% $fT>MIC$

<table>
<thead>
<tr>
<th>MIC mg/L</th>
<th>Probability of target attainment</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1 mg/L</td>
<td>no difference ESBL and non-ESBL producers</td>
</tr>
<tr>
<td>2-4 mg/L</td>
<td>variable successful outcomes</td>
</tr>
<tr>
<td>&gt;4 mg/L</td>
<td>poor outcomes</td>
</tr>
</tbody>
</table>

EUCAST decreased ceftazidime and cefepime breakpoints due to evidences on clinical and MIC correlations:

- ≤1 mg/L: no difference ESBL and non-ESBL producers
- 2-4 mg/L: variable successful outcomes
- >4 mg/L: poor outcomes

Paterson et al. JCM 2001; 3; 9:2206-12; Andes & Craig. CMI 2005; 11 (Suppl. 6):10-7
Bin et al. DMID 2006; 56:351-7; Bhat et al. AAC 2007; 51:4390-5
The current situation on Enterobacteriaceae …

<table>
<thead>
<tr>
<th></th>
<th>FDA</th>
<th>CLSI (2011)</th>
<th>EUCAST (EMA) (2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td><strong>Imipenem</strong></td>
<td>≤4</td>
<td>≤1 (4)*</td>
<td>≥4 (16)</td>
</tr>
<tr>
<td><strong>Meropenem</strong></td>
<td>≤4</td>
<td>≤1 (4)</td>
<td>≥4 (16)</td>
</tr>
<tr>
<td><strong>Ertapenem</strong></td>
<td>≤2</td>
<td>≤0.25 (2)</td>
<td>≥1 (8)</td>
</tr>
<tr>
<td><strong>Doripenem</strong></td>
<td>≤0.5</td>
<td>≤1 (ND)</td>
<td>≥4 (ND)</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>R</td>
<td>ECOFF</td>
</tr>
<tr>
<td></td>
<td>≤2</td>
<td>&gt;8</td>
<td>≤0.5; ≤1**</td>
</tr>
<tr>
<td></td>
<td>≤2</td>
<td>&gt;8</td>
<td>≤0.125</td>
</tr>
<tr>
<td></td>
<td>≤0.5</td>
<td>&gt;1</td>
<td>≤0.06</td>
</tr>
<tr>
<td></td>
<td>≤1</td>
<td>&gt;4</td>
<td>≤0.12</td>
</tr>
</tbody>
</table>

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

EUCAST breakpoint are higher than those of CLSI!
CLSI & EUCAST carbapenem clinical breakpoints

**CLSI**
- New breakpoints published in June 2010 and January 2011*
  - to capture carbapenemase (mainly KPCs) producers
  - Rationale:
    - Pk/Pd tools avoiding PK subject variability *(inflated variance)*
- Modified Hodge test no longer necessary unless for infection control and epidemiological purposes

*Documents M100-S20-U; M100-S21

**EUCAST**
- Breakpoints published in 2006 and with doripenem in 2008*
  - define as “clinical breakpoints” not to detect carbapenemases
  - Rationale:
    - MIC distribution of wild-type isolates, MBL-KPC producers
    - Pk/Pd data
    - Review of clinical data
- Carbapenemase detection no longer necessary for clinical categorization unless for infection control purposes

*Version 1.3, January 2011
### Carbapenem breakpoints and Enterobacteriaceae

**IMIPENEM***

<table>
<thead>
<tr>
<th>Breakpoints prior to harmonisation (mg/L) S ≤&lt;(R) &gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species related breakpoints</strong></td>
</tr>
<tr>
<td><strong>Staphylococcus spp.</strong></td>
</tr>
<tr>
<td>4/4</td>
</tr>
<tr>
<td><strong>Streptococcus spp.</strong></td>
</tr>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
</tr>
<tr>
<td><strong>Enterococcus spp.</strong></td>
</tr>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
</tr>
<tr>
<td><strong>Pseudomonas spp.</strong></td>
</tr>
<tr>
<td><strong>Acinetobacter spp.</strong></td>
</tr>
<tr>
<td><strong>Haemophilus spp.</strong></td>
</tr>
<tr>
<td><strong>Moraxella spp.</strong></td>
</tr>
<tr>
<td><strong>Neisseria meningitidis</strong></td>
</tr>
<tr>
<td><strong>Neisseria gonorrhoeae</strong></td>
</tr>
<tr>
<td><strong>Pasteurella multocida</strong></td>
</tr>
<tr>
<td><strong>Gram-negative anaerobes</strong></td>
</tr>
<tr>
<td><strong>Campylobacter spp.</strong></td>
</tr>
</tbody>
</table>

**EUCAST,** \( S \leq 2 \) \( R: >8 \)

*Imipenem Rationale Document, 2009*
Probability of target attainment (PTA) for Enterobacteriaceae for 500 mg x 4 i.v.

- PTA: \( fT>MIC \) for 2 log experimental reduction = 35-55; \( fT>MIC \) from clinical data = 54
- Pk parameters used to obtained the PTA:
  - Volume of distribution (Vd): 1.5 L, CV 15%
  - Elimination half life (t): 1.05h, CV 20%
  - Fraction unbound (Fu): 80%
  - Infusion time: 0.5 h

EUCAST RD: Imipenem (Monte Carlo simulation)
What has been the impact of these “new” breakpoints?

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

- critical voices alerting on negative consequence for no further detection and report of ESBLs and carbapenemases
  Livermore et al. JAC 2012; 67:1569-77

- analysis and meta-analysis of different impact on mortality of ESBL and carbapenemase producing organisms
  Bonten et al. JAC 2012; 67:1311-20; Falagas et al. AAC 2012; 4214-22
What has been the impact of these “new” breakpoints?

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

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

- major impact when using CLSI than EUCAST
- major impact for ceftazidime and cefepime than for cefotaxime
- geographic dependent impact (different ESBL epidemiology)
- origin (hospital or community-onset) dependent impact
% of ESBL-\textit{E. coli} isolates susceptible to 3\textsuperscript{rd} / 4\textsuperscript{th} gen. ceph. when using CLSI and EUCAST breakpoints in different studies

- **Spain**
  - Rodriguez-Baño (CLSI): 52%
  - Hoban (CLSI): 38%
- **USA**
  - Chen (CLSI): 19%
- **Asia**
  - Chen (CLSI): 7.5%
  - FEP: 8.7%
- **Spain**
  - Rodriguez-Baño (EUCAST): 14.714.7%
3rd/4th gen. cephalosporin breakpoints in Enterobacteriaceae

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

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

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

**References:**
Willamson et al. EJCMID 2012; 31:821-4
1.- Similar number of **clinical cases** on record where cephalosporins and carbapenems have proved effective and ineffective against infections due to low-MIC ESBL and carbapenemase producers, respectively.

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

3.- Although breakpoint committees advocate ESBL and carbapenemase detection for epidemiological purposes, some **laboratories will abandon seeking these enzymes** for treatment purposes, leading to a loss of critical infection control information.
Clinical data on outcome for ESBL L producers indicates that outcome decrease when MICs are > 2 mg/L.

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

Paterson et al. JCM 2001; 39:2206-12

Andes & Craig. CMI 2005; 11 (Suppl. 6):10-7
Impact of antibiotic MIC on infection outcome in patients with susceptible Gram-negative bacteria

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

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

ESBL production in Enterobacteriaceae bacteremia is associated with higher mortality (OR 2.35; 95% CI, 1.90-2.91), although it is reduced after adjustment by inadequate empirical therapy.

Carbapenem breakpoints in Enterobacteriaceae

- Survival probability (Kaplan-Meier curves) of patients with VIM-producing *K. pneumoniae* bloodstream infections according with susceptibility to carbapenems (imipenem or meropenem):

Patients infected with a VIM-(+) organism for which the MICs of both imipenem and meropenem were >4 mg/L were more likely to die than those infected with a VIM-(+) carbapenem-susceptible organism (P 0.044)

Not all patients were treated with carbapenems

Clinical correlation of carbapenem treatment in monotherapy

![](chart)

- **≤0.5**: 70% efficacy
- **≤4**: 60% efficacy
- **8**: 50% efficacy
- **>8**: 40% efficacy

22 patients with *K. pneumoniae* without carbapenemase

44 patients with *K. pneumoniae* with VIM, NDM or KPC

---

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

<table>
<thead>
<tr>
<th>Antibiotic regimen</th>
<th>No. of patients (%)</th>
<th>Outcome success (%)</th>
<th>Failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monotherapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>64 (24.2)</td>
<td>35 (54.7)</td>
<td>29 (45.3)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>8 (4.7)</td>
<td>5 (62.5)</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>16 (6.8)</td>
<td>12 (75.0)</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td><strong>Carbapenem</strong></td>
<td>23 (9.8)</td>
<td>18 (78.3)</td>
<td>5 (21.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>111 (47.5)</td>
<td>70 (63.1)</td>
<td>41 (36.9)</td>
</tr>
</tbody>
</table>

| Combination therapy              |                     |                     |             |
| Two or more active drugs         | 52 (22.2)           | 38 (73.1)           | 14 (26.9)   |
| (carbapenem not included)        |                     |                     |             |
| Two or more active drugs         | 30 (12.8)           | 28 (93.3)           | 2 (6.7)     |
| (carbapenem included)            |                     |                     |             |
| **Total**                        | 82 (35.0)           | 66 (80.5)           | 16 (19.5)   |
| ‘Inappropriate’ therapy          | 41 (17.5)           | 23 (56.1)           | 18 (43.9)   |
| **Total**                        | 234 (100)           | 159 (67.9)          | 75 (32.1)   |
Mortality in bloodstream infections and KPC-K. pneumoniae

- Higher mortality (30-day) rate in patients treated with monotherapy (54.3%) that those with combination (34.1%) therapy ($P=0.02$).

- Significant decreased of mortality in patients treated with combination therapy including meropenem.

- Kaplan-Meier curves (survival)

- Mortality (%): combination therapy

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

- MIC (mg/L)
- %
- Nonsurvivors
- Survivors

**EUCAST expert rules v2: major modifications**

**β-lactams and *Haemophilus influenzae***

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

<table>
<thead>
<tr>
<th>Agents tested</th>
<th>Agents affected</th>
<th>Rule</th>
<th>Exceptions, scientific basis and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin or amoxicillin (and β-lactamase detection)</td>
<td>Ampicillin, amoxicillin and piperacillin</td>
<td>IF β-lactamase positive THEN report as R to ampicillin, amoxicillin and piperacillin</td>
<td>Ampicillin is the class representative for amoxicillin. Resistance to ampicillin by production of β-lactamase may be misidentified by the disk diffusion technique. Production of β-lactamase should be examined with a chromogenic test.</td>
</tr>
</tbody>
</table>
EUCAST expert rules v2: major modifications

β-lactams and *Haemophilus influenzae*

Rule 10.2 v2 (evidence grade C)

<table>
<thead>
<tr>
<th>Agents tested</th>
<th>Agents affected</th>
<th>Rule</th>
<th>Exceptions, scientific basis and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin or amoxicillin (and β-lactamase detection)</td>
<td>Ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin and piperacillin-tazobactam.</td>
<td>IF BLNAR THEN report as R to ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, cefaclor, cefuroxime and cefuroxime axetil.</td>
<td>BLNAR isolates have reduced affinity of PBPs for β-lactams. Although piperacillin and piperacillin-tazobactam appear less affected by the PBP-mediated resistance mechanisms evidence regarding clinical efficacy is lacking.</td>
</tr>
</tbody>
</table>

BLNAR: β-lactamase negative but ampicillin resistant
## EUCAST expert rules v2: major modifications

### β-lactams and *Haemophilus influenzae*

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

<table>
<thead>
<tr>
<th>Agents tested</th>
<th>Agents affected</th>
<th>Rule</th>
<th>Exceptions, scientific basis and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-clavulanate (and β-lactamase detection)</td>
<td>Ampicillin- sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin and piperacillin-tazobactam.</td>
<td>IF BLPACR THEN report as R to amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, piperacillin, piperacillin-tazobactam. cefuroxime and cefuroxime axetil.</td>
<td>BLPACR isolates produce β-lactamase and have reduced affinity of PBPs for β-lactams. Although piperacillin and piperacillin-tazobactam appear less affected by the PBP-mediated resistance mechanisms evidence regarding clinical efficacy is lacking.</td>
</tr>
</tbody>
</table>

**BLPACR**: β-lactamase positive and amoxicillin-clavulanate resistant
EUCAST expert rules v2: major modifications

β-lactams and *Haemophilus influenzae*: practical issues

- Test for β-lactamase and report positive isolates R to penicillins without β-lactamase inhibitors
- Use ampicillin and amoxicillin breakpoints only to β-lactamase negative isolates
- Isolates may be R to penicillins, aminopenicillins and/or cephalosporins due to changes in PBPs (BLNAR)
- A few β-lactamase positive isolates may have also PBP changes (BLPACR)
- Isolates S to ampicillin and amoxicillin are also S to amoxicillin-clavulanate, piperacillin and piperacillin-tazobactam
- Isolates S to amoxicillin-clavulanate are also S to piperacillin tazobactam
EUCAST expert rules v2: major modifications

β-lactamase test

Positive

Ampicillin-R (BLP)

Expert rule 10.1
Report R to:
Ampicillin
Amoxicillin
Piperacillin

Amox/clav-R (BLPACR)

Expert rule 10.3
Report R to:
Ampicillin
Amoxicillin
Amox/clav
Amp/sulb
Piperacillin
Pip/tazb
Cefaclor
Cefuroxime

Ampicillin-R (BLNAR)

Expert rule 10.2
Report R to:
Ampicillin
Amoxicillin
Amox/clav
Amp/sulb
Piperacillin
Piper/tazb
Cefaclor
Cefuroxime

Negative

Ampicillin-S (BLN)

Benzylpenicillin
1 unit screen test
EUCAST expert rules v2: major modifications

Benzylpenicillin - 1 unit screen test

- **S ≥12 mm**
  - Ampicillin-S (BLN)
  - Expert rule 10.2
    - Report R to:
      - Ampicillin
      - Amoxicillin
      - Amox/clav
      - Amp/sulb
      - Piperacillin
      - Piper/tazb
      - Cefaclor
      - Cefuroxime

- **R < 12 mm**
  - β-lactamase test
    - Negative
      - Ampicillin-R (BLNAR)
    - Positive
      - Ampicillin-R Amox/clav-S (BLP)
      - Ampicillin-R Amox/clav-R (BLPACR)

  - Expert rule 10.1
    - Report R to:
      - Ampicillin
      - Amoxicillin
      - Piperacillin

  - Expert rule 10.3
    - Report R to:
      - Ampicillin
      - Amoxicillin
      - Amox/clav
      - Amp/sulb
      - Piperacillin
      - Pip/tazb
      - Cefaclor
      - Cefuroxime
### EUCAST expert rules v2

**Salmonella spp. and fluoroquinolones**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Agents tested</th>
<th>Agents affected</th>
<th>Rule</th>
<th>Exceptions, scientific basis and comments</th>
<th>Evidence grade</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Ciprofloxacin</td>
<td>All fluoroquinolones</td>
<td>IF ciprofloxacin MIC &gt; 0.06 mg/L, THEN report as resistant to all fluoroquinolones.</td>
<td>Evidence for clinical failure of fluoroquinolones in case of resistance due to the acquisition of at least one target mutation in <em>gyrA</em>.</td>
<td>A (Salmonella typhi) B (other <em>Salmonella</em> spp.)</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
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.

Future modification of fluoroquinolones breakpoints?
Salmonella spp., nalidixic acid and fluoroquinolones
EUCAST expert rules in antimicrobial susceptibility testing

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