EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

www.EUCAST.org

# EXPERT RULES IN SUSCEPTIBILITY TESTING – RATIONALE, ADVANTAGE AND DISADVANTAGES



SaludMadrid
Dr. Rafael Cantón
Hospital Universitario Ramón y Cajal
SERVICIO DE MICROBIOLOGÍA Y PARASITOLOGÍA

instituto ramón y cajal de investigación sanitaria irycis

Departamento de Microbiología II
Universidad Complutense. Madrid

# EUCAST expert rules

- **Expert rules** in **antimicrobial susceptibility testing (AST)**

  - describe actions to be taken on the basis of specific AST results

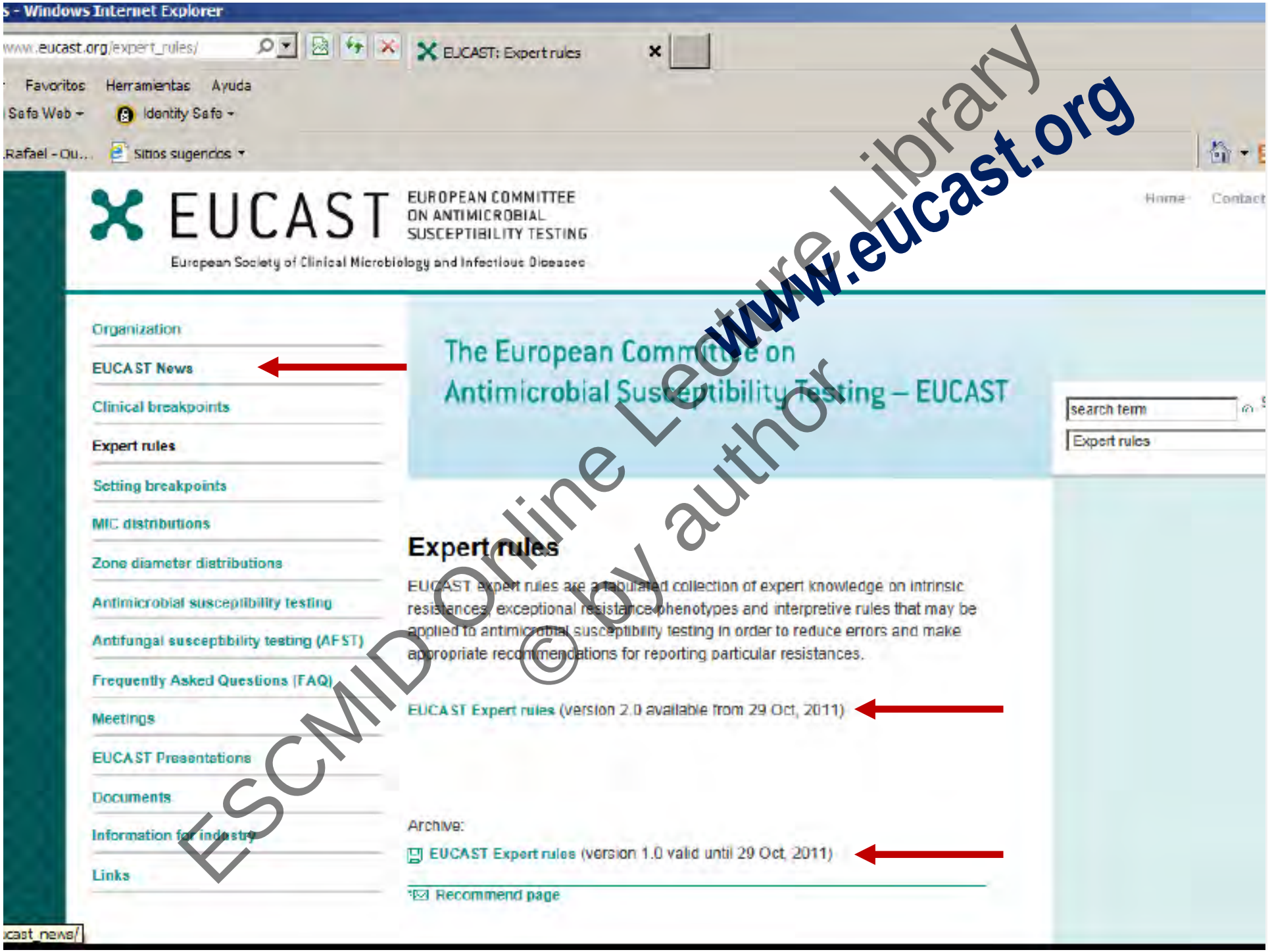
  - based on clinical breakpoints & resistance mechanism knowledge

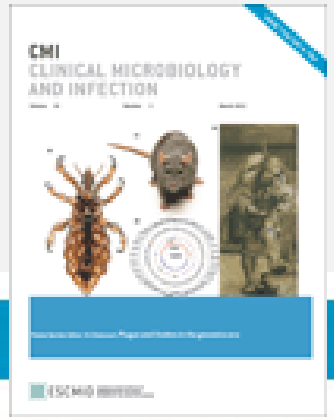
  - assist clinical microbiologists in the interpretation of AST results

  - 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
Leclercq R et al. Clin Microbiol Infect 2011 Oct 21 [Epub ahead of print]
http://www.eucast.org








REVIEW

10.1111/j.1469-0691.2011.03703.x

# EUCAST expert rules in antimicrobial susceptibility testing

**R. Leclercq[1,2], R. Cantón[2,3,4], D. F. J. Brown[4], C. G. Giske[2,4,5], P. Heisig[2,6], A. P. MacGowan[4,7], J. W. Mouton[4,8], P. Nordmann[2,9], A. C. Rodloff[4,10], G. M. Rossolini[2,11], C.-J. Soussy[4,12], M. Steinbakk[4,13], T. G. Winstanley[2,14] and G. Kahlmeter[4,15]**

*1) Laboratoire de Microbiologie, CHU Côte de Nacre, Caen, France, 2) EUCAST Subcommittee on Expert Rules, 3) Servicio de Microbiología and CIBER en Epidemiología y Salud Pública (CIBERESP), Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain, 4) EUCAST Steering Committee, 5) Clinical Microbiology, MTC-Karolinska Institutet, Karolinska University Hospital, Solna, Sweden, 6) Department of Pharmacy, Biology & Microbiology, University of Hamburg, Hamburg, Germany, 7) Department of Medical Microbiology, Southmead Hospital, Bristol, UK, 8) Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherland, 9) Service de Bactériologie-Virologie, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France, 10) Institut fur Medizinische Mikrobiologie der Universitat Leipzig, Leipzig, Germany, 11) Dipartimento di Biotecnologie, Sezione di Microbiologia, Siena, Italy, 12) Hôpital Henri Mondor, Service de Bactériologie, Creteil, France, 13) Department of Bacteriology and Immunology, Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway, 14) Department of Microbiology, Royal Hallamshire Hospital, Sheffield, UK and 15) Clinical Microbiology, Central Hospital, Växjö, Sweden*



***Clin Microbiol Infect, 2011 Oct 21 [Epub ahead of print]***

# EUCAST expert rules

- The **EUCAST expert rules** in antimicrobial susceptibility testing are divided into:

  **- intrinsic resistances**

  **- exceptional phenotypes**

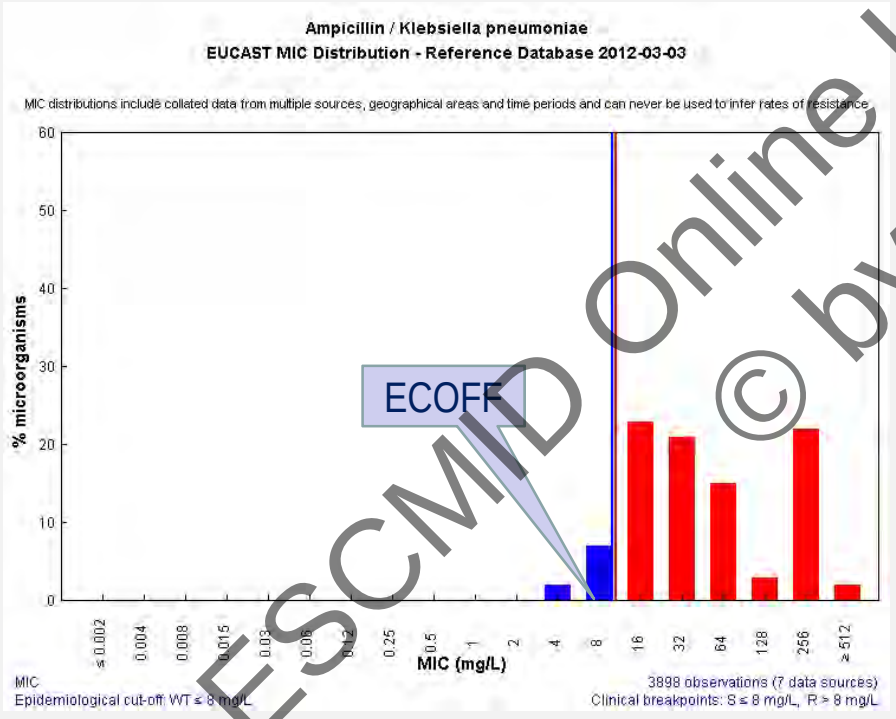
  **- interpretive rules**

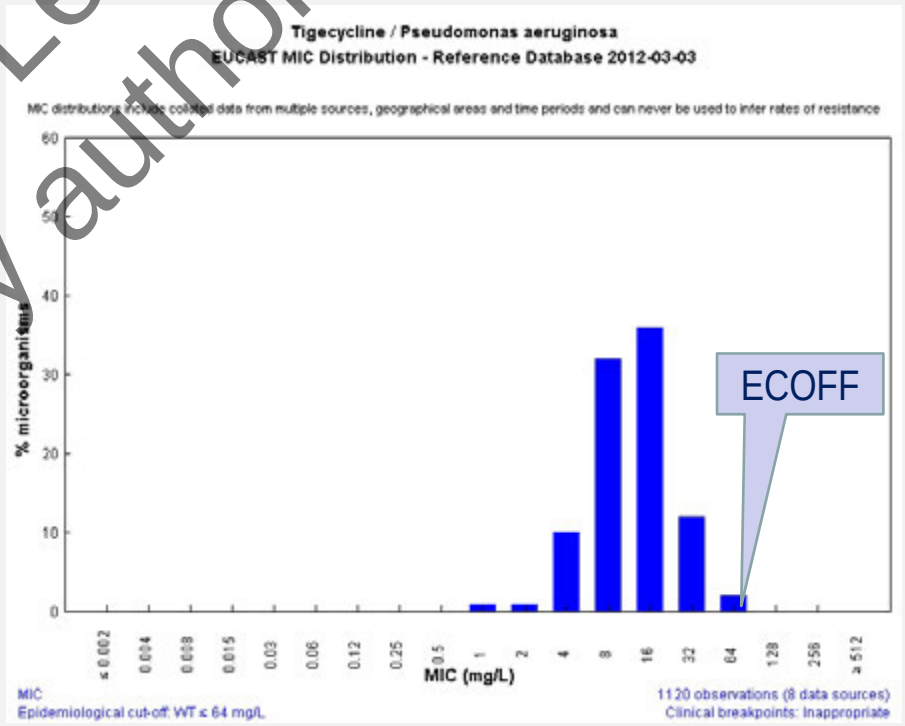


Leclercq R et al. Clin Microbiol Infect 2011 Oct 21 [Epub ahead of print]
http://www.eucast.org

# EUCAST expert rules *v2:* intrinsic resistance

- Characteristic of all or almost all isolates of the bacterial specie
- The antimicrobial activity of the drug is clinically insufficient or antimicrobial resistance is innate, rendering it clinically useless
- Antimicrobial susceptibility is unnecessary

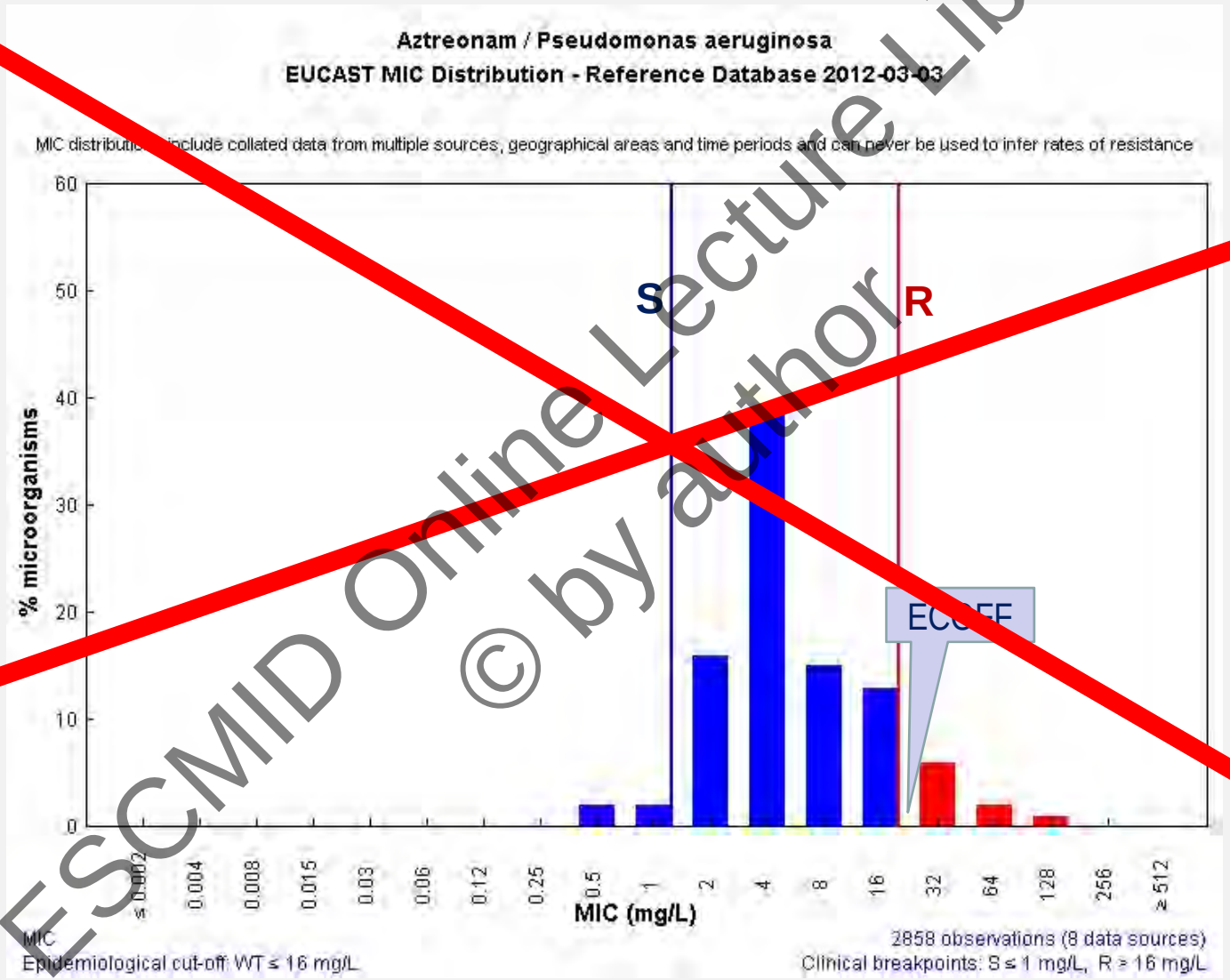





www.eucast.org

# EUCAST expert rules *v2:* intrinsic resistance



# EUCAST expert rules *v2:* exceptional phenotypes

- Phenotypes of resistance of bacterial species to particular antimicrobial agents that have not yet reported or are very rare
- They may change over time and should be define locally

| Rule no. | Organisms | Exceptional phenotypes |
|---|---|---|
| 5.1 | Any *Enterobacteriaceae* (except *Proteae*) | Resistant to meropenem and/or imipenem[a] |
| 5.2 | *Serratia marcescens* and *Proteae* | Susceptible to colistin |
| 5.3 | *Pseudomonas aeruginosa* and *Acinetobacter* spp. | Resistant to colistin |
| 5.4 | *Haemophilus influenze* | Resistant to any third-generation cephalosporin, carbapenems, and fluoroquinolones |
| 5.5 | *Moraxella catarrhalis* | Resistant to ciprofloxacin and any third-generation cephalosporin |
| 5.6 | *Neisseria meningitidis* | Resistant to any third-generation cephalosporin and fluoroquinolones |
| 5.7 | *Neisseria gonorrhoeae* | Resistant to third-generation cephalosporin and spectinomycin |

[a]Except in countries in which carbapenemase-producing *Enterobacteriaceae* are not rare.

# EUCAST expert rules *v2:* interpretive rules

- 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 β-lactams | 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, except those specifically licensed to treat infections caused by methicillin-resistant staphylococci due to low affinity for PBP2a | Production of PBP2a (encoded by *mecA*) leads to cross resistance to β-lactams except ceftobiprole and ceftaroline. | A | Page et al. 2006 Chambers et al. 1990 |

IF ... THEN ...

# EUCAST expert rules *v2:* interpretive rules

***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

Leclercq R et al. Clin Microbiol Infect 2011 Oct 21 [Epub ahead of print]
http://www.eucast.org

# EUCAST expert rules *v2:* major modifications

## β-lactam antibiotics

- **Deletion of ESBL expert rule from *v1***

  - ESBL detection and clinical category modification in extended spectrum cephalosporins no longer exist (*report as found*)

.

The cephalosporin breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including ESBL and plasmid mediated AmpC). Some strains that produce β-lactamases are S or I to 3rd or 4th gen. cephalosporins with these breakpoints and **should be reported as found**, i.e. the presence or absence of an ESBL does not in itself influence the categorization of susceptibility. **In many areas, ESBL detection and characterization is recommended or mandatory for infection control purposes**

EUCAST Clinical Breakpoint Table v. 2.0, valid from 2012-01-01

# EUCAST expert rules *v2*

## Q1

# EUCAST expert rules *v2*

## Q2

# CLSI & EUCAST carbapenem clinical breakpoints

- *The current situation on Enterobacteriaceae…*

| | FDA | CLSI (2011) | | EUCAST (EMA) (2011) | | |
|---|---|---|---|---|---|---|
| | S | S | R | S | R | ECOFF |
| **Imipenem** | **≤4** | **≤1** (4)* | **≥4** (16) | **≤2** | **>8** | **≤0.5; ≤1**** |
| **Meropenem** | **≤4** | **≤1** (4) | **≥4** (16) | **≤2** | **>8** | **≤0.125** |
| **Ertapenem** | **≤2** | **≤0.25** (2) | **≥1** (8) | **≤0.5** | **>1** | **≤0.06** |
| **Doripenem** | **≤0.5** | **≤1** (ND) | **≥4** (ND) | **≤1** | **>4** | **≤0.12** |

*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

# EUCAST expert rules *v2:* major modifications

## Consequences of new edited breakpoints

- Deletion of expert rules on carbapenemes from version 1

| Rule no. | Organisms | Agent | Rule | Exceptions | Scientific basis | Evidence Grade |
|---|---|---|---|---|---|---|
| 9.6 | Enterobacteriaceae, *Acinetobacter* spp., *Pseudomonas* spp. | Carbapenems | Test results re[illegible]ding one carbapenem (imip[illegible]em, meropenem, ertapen[illegible] cann[illegible] be extrapolated to the ot[illegible] carbapenems. | *Ent[illegible]obacteriaceae* only: If [illegible]sistant to either imipenem, meropenem, report as resistant to ertapenem without further testing. | There is variable stability to AmpC hydrolysis, dependence of porins and susceptibility to the efflux pumps. | C |

# EUCAST expert rules *v2:* major modifications

## Consequences of new edited breakpoints

- Deletion of expert rules from version 1 on carbapenemes

| Rule no. | Organisms | Agent | Rule | Scientific basis | Evidence Grade |
|---|---|---|---|---|---|
| 9.7 | Enterobacteriaceae, *Acinetobacter* spp., *Pseudomonas* spp. | Carbapenems | If production of metallo-β-lactamase is confirmed, report the susceptible results as intermediate and the intermediate results as resistant for any β-lactam except aztreonam which should be reported as found. | Metallo-β-lactamases can hydrolyse all β-lactams except monobactams. | B |
| 9.8 | Enterobacteriaceae | Carbapenems, oxyimino cephalosporins, aztreonam | If reduced susceptibility to carbapenems AND oxyimino cephalosporins AND aztreonam, resistance may reflect either KPC, GES β-lactamases or combinations of AmpC or ESBL plus impermeability. In either case ertapenem tends to be the most affected carbapenem. Synergy between carbapenems and clavulanate may arise with either KPC enzymes or with combinations of ESBL and impermeability. | KPC carbapenemase or combinations of ESBL or AmpC and impermeability. | C |

## EUCAST Version 2.0, January 2012

## Breakpoint table for interpretation or MICs and zone diameters

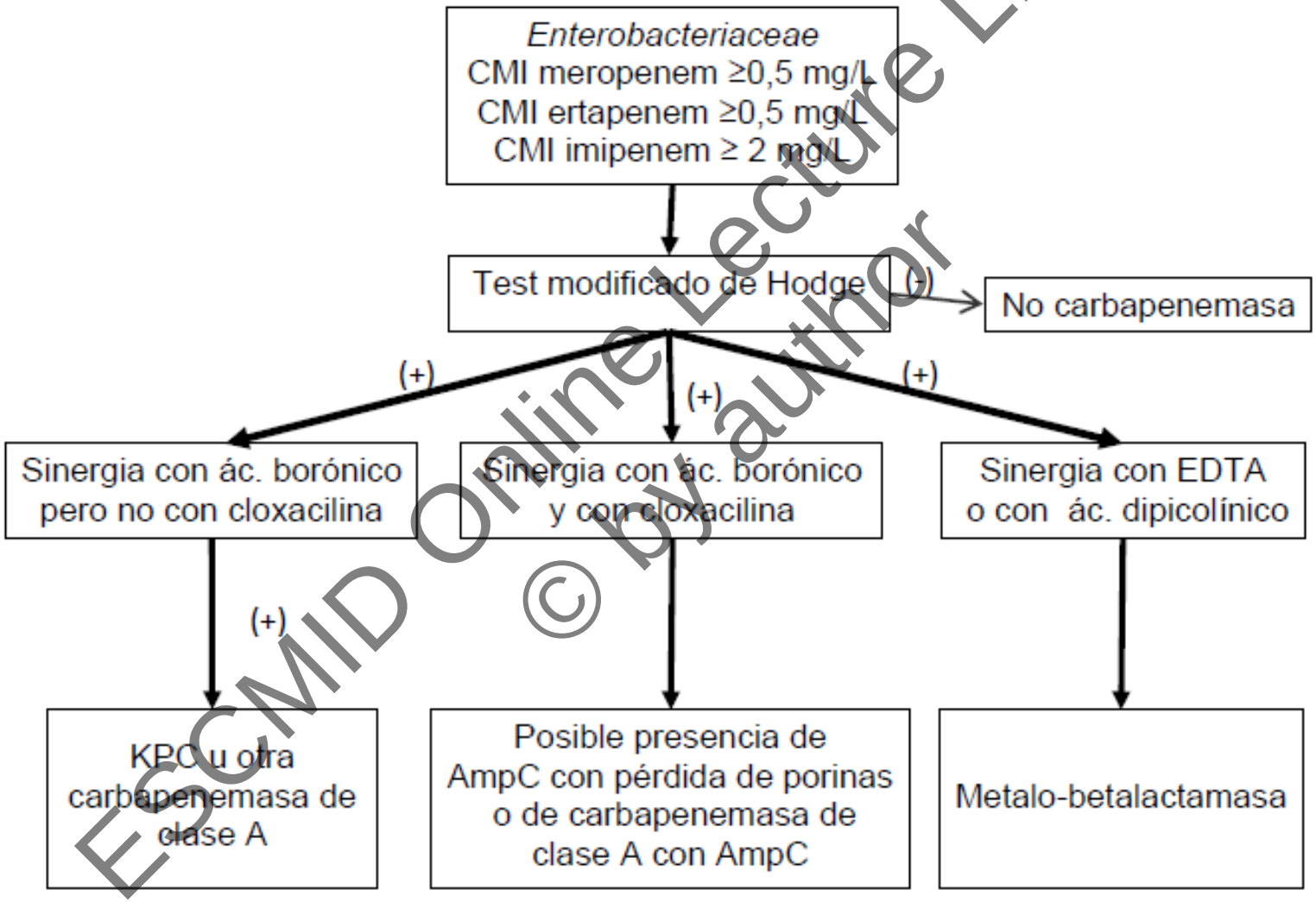
| Carbapenems[1] | MIC breakpoint (mg/L) | |
|---|---|---|
| | S ≤ | R > |
| Doripenem | 1 | 4 |
| Ertapenem | 0.5 | 1 |
| Imipenem[2] | 2 | 8 |
| Meropenem | 2 | 8 |

Low-level resistance is common in *Morganella spp., Proteus spp. and Providencia spp.*

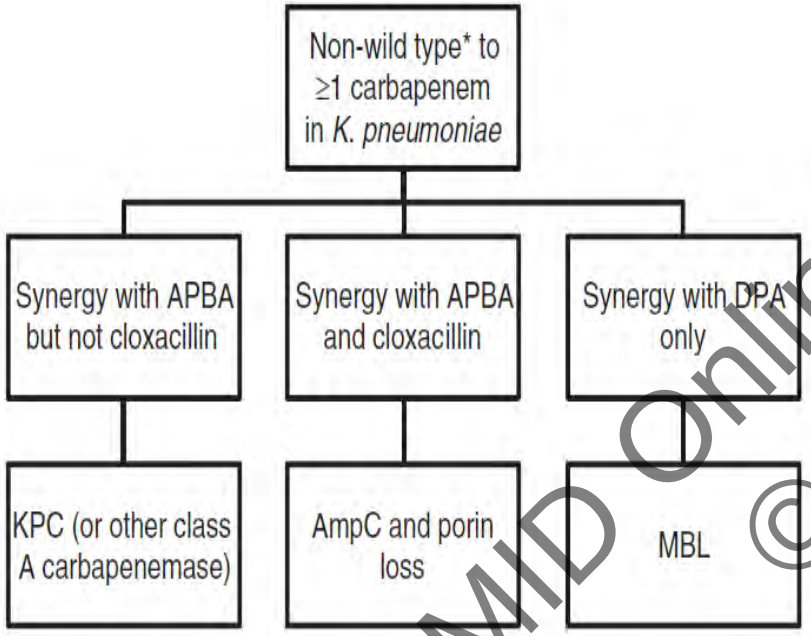
The carbapenem breakpoints for Enterobacteriaceae will detect all clinically important resistance mechanisms (including the majority of carbapenemases). Some isolates that produce carbapenemase are categorised as S with these breakpoints and **should be reported as tested,** i.e. the presence or absence of a carbapenemase does not in itself influence the categorisation of susceptibility. **In many areas, carbapenemase detection and characterisation is recommended or mandatory for infection control purposes.**

# Procedimientos en Microbiología Clínica

Recomendaciones de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica



**Giske et al.** A sensitive and specific phenotypic assay for detection of metallo-β-lactamases and KPC in *K. pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. **Clin Microbiol Infect 2011;17:552-6**




| Test | β-Lactamases sought by test(s) | Sensitivity (%) | Specificity (%) |
|---|---|---|---|
| APBA-positive, cloxacillin-negative | KPC | 100 | 98 |
| APBA-positive, cloxacillin-positive | AmpC[a] | 80 | 100 |
| EDTA-positive | MBL | 100 | 88 |
| DPA-positive | MBL | 100 | 100 |
| Positive cloverleaf test result | KPC, MBL, OXA-48 | 100 | 78 |

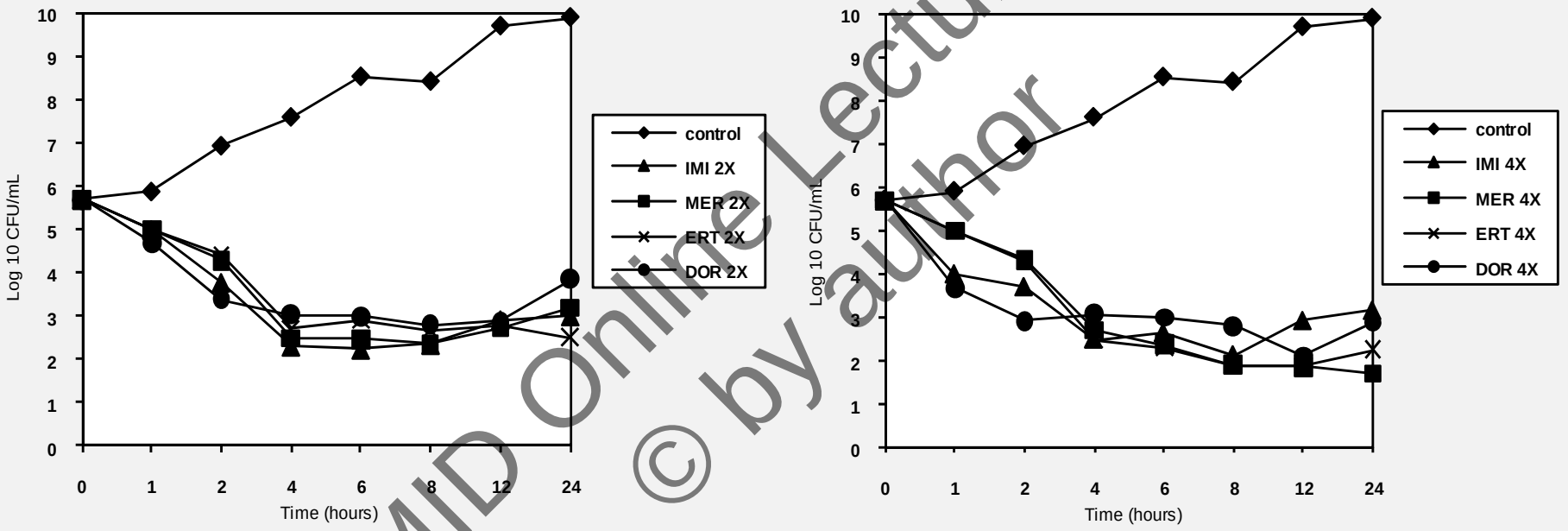
MBL, metallo-β-lactamase.
[a]Combination of AmpC hyperproduction and porin loss.

**Grundmann et al.** Working Group. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. **Euro Surveill 2010;15(46):pii=19711**

# Carbapenem breakpoints and Enterobacteriaceae

- Bactericidal activity against VIM-1-producing *K. pneumoniae*

  MIC: imipenen, meropenem, doripenem = 8 mg/L, ertapenem = 1 mg/L




Morosini et al. 2011

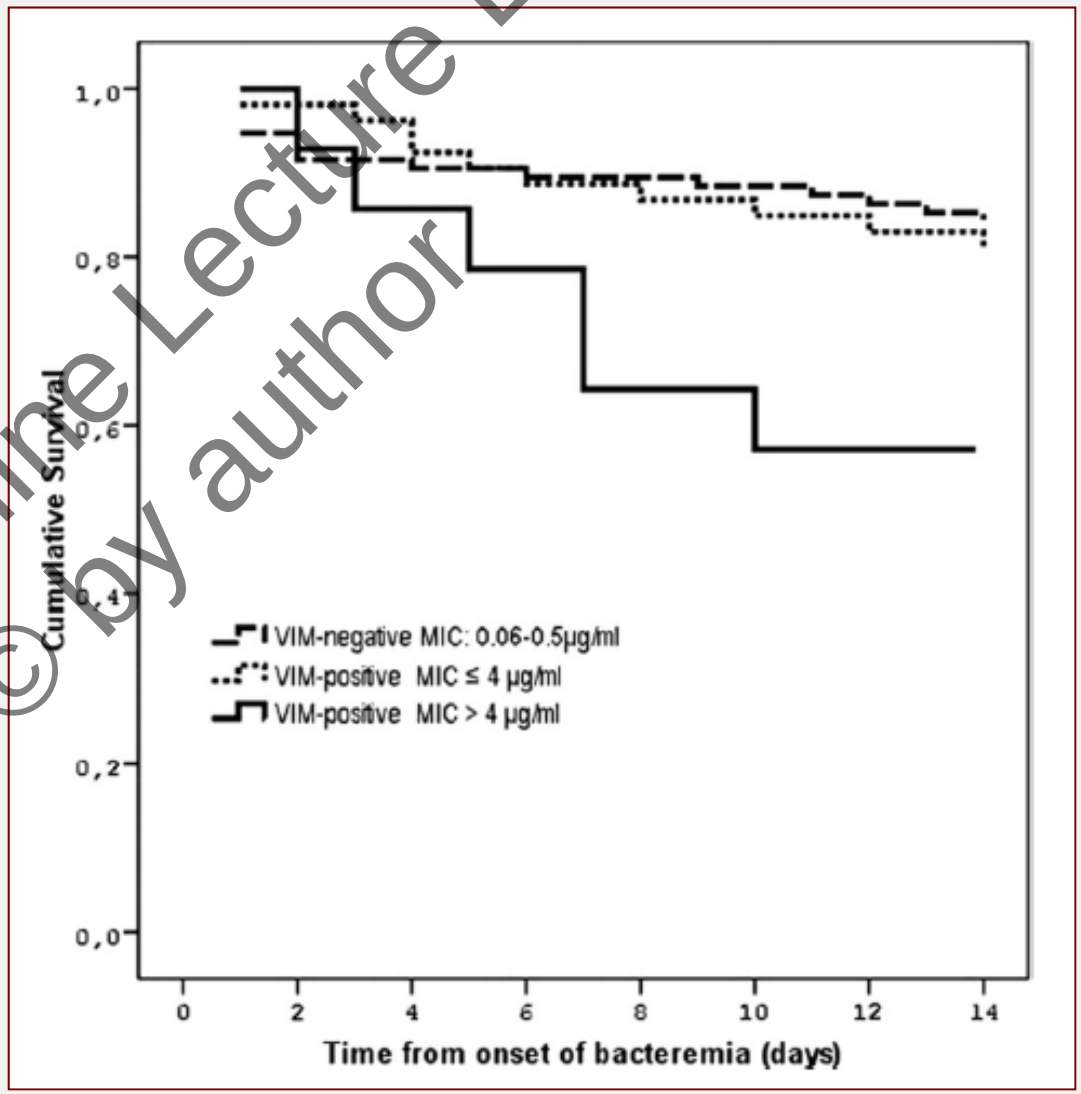
- Presence of KPC in Enterobacteriaceae exhibiting carbapenem MICs between 1-16 mg/L had no impact on the PD (%T > MIC) necessary for bacteriostasis by carbapenems

Craig et al. 48th ICAAC, 2008, abstract A-029

# Imipenem / meropenem and metalo-β-lactamase (VIM)

- Survival probability of patients with VIM-producing *K. pneumoniae* blood stream infections according with susceptibility to carbapenems

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 of VIM-(-) organisms (P 0.044)

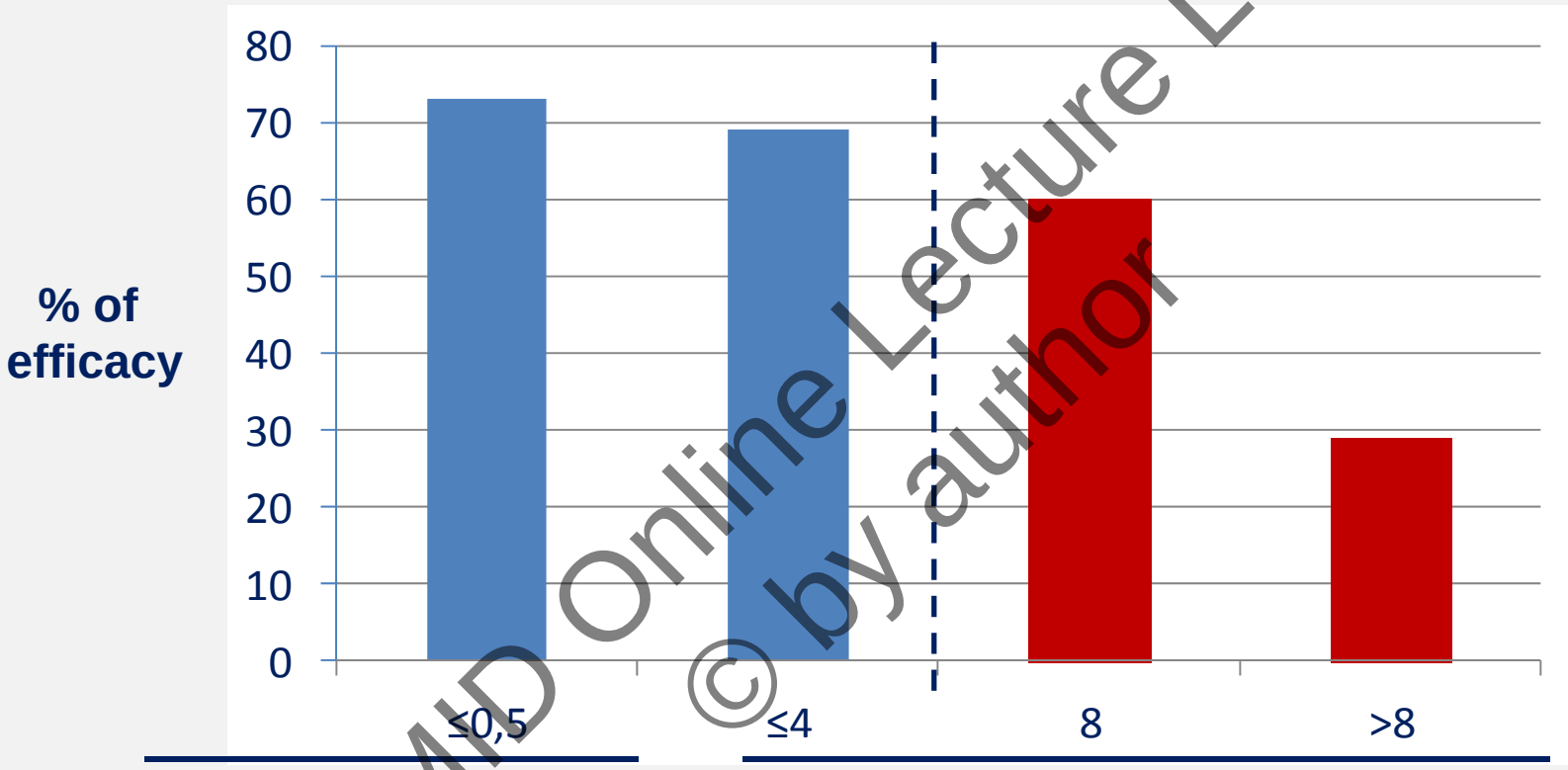



**Not all patients were treated with carbapenems**

Daikos et al. Antimicrob Agents Chemother 2009; 53: 1868-73

# Carbapenemase isolates and carbapenem treatment

## Clinical outcomes of carbapenem monotherapy treatment




22 patients with non-carbapenemase-producing *K. pneumoniae* isolates

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

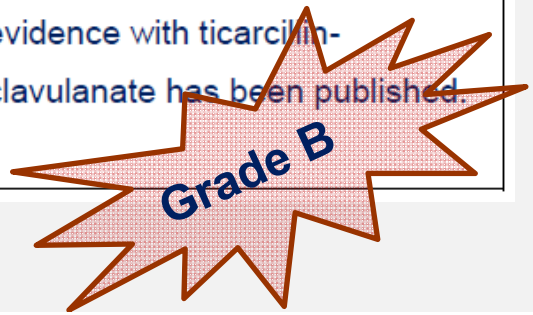


Daikos et al.Clin Microbiol Infect 2011; 17: 1135-41

# EUCAST expert rules *v2:* major modifications

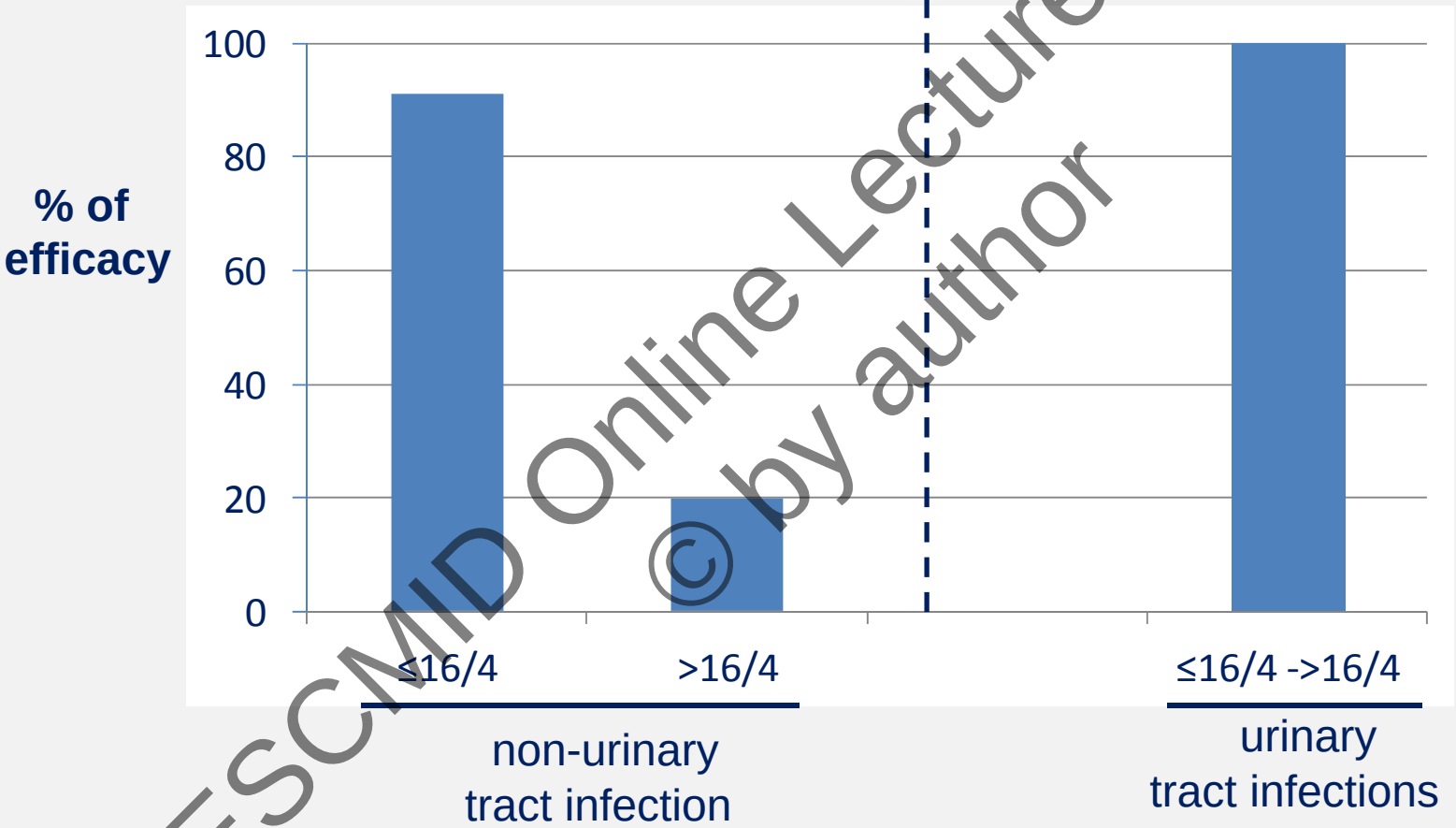
## β-lactam-β-lactamase inhibitor combinations and Enterobacteriaceae (expert rule 9.1)

| Agents tested | Agents affected | Rule | Exceptions, scientific basis and comments |
|---|---|---|---|
| Cefotaxime, ceftriaxone, ceftazidime, cefepime, amoxicillin-clavulanate, ampicillin- sulbactam, piperacillin-tazobactam | Amoxicillin-clavulanate, ampicillin-sulbactam piperacillin-tazobactam | **IF I or R to any 3rd gen. (cefotaxime, ceftriaxone, ceftazidime) or 4th gen. (cefepime) oxyimino-cephalosporin, AND susceptible to amoxicillin-clavulanate, ampicillin-sulbactam or piperacillin-tazobactam THEN report as tested and enclose a warning on uncertain therapeutic outcome for infections other than urinary tract infections.** | ESBL producers are often categorized as S to combinations of a penicillin plus a β-lactamase inhibitor. With the exception of urinary tract infections and blood stream infections secondary to this origin, the use of these combinations in infections caused by ESBL producers remains controversial, and should be approached with caution. No evidence with ticarcillin-clavulanate has been published. |



# β-lactam - β-lactamase inhibitor combinations

Clinical outcomes of **piperacillin-tazobactam** (alone or in combination for at least 72 h) in infection caused by ESBL-Enterobacteriaceae

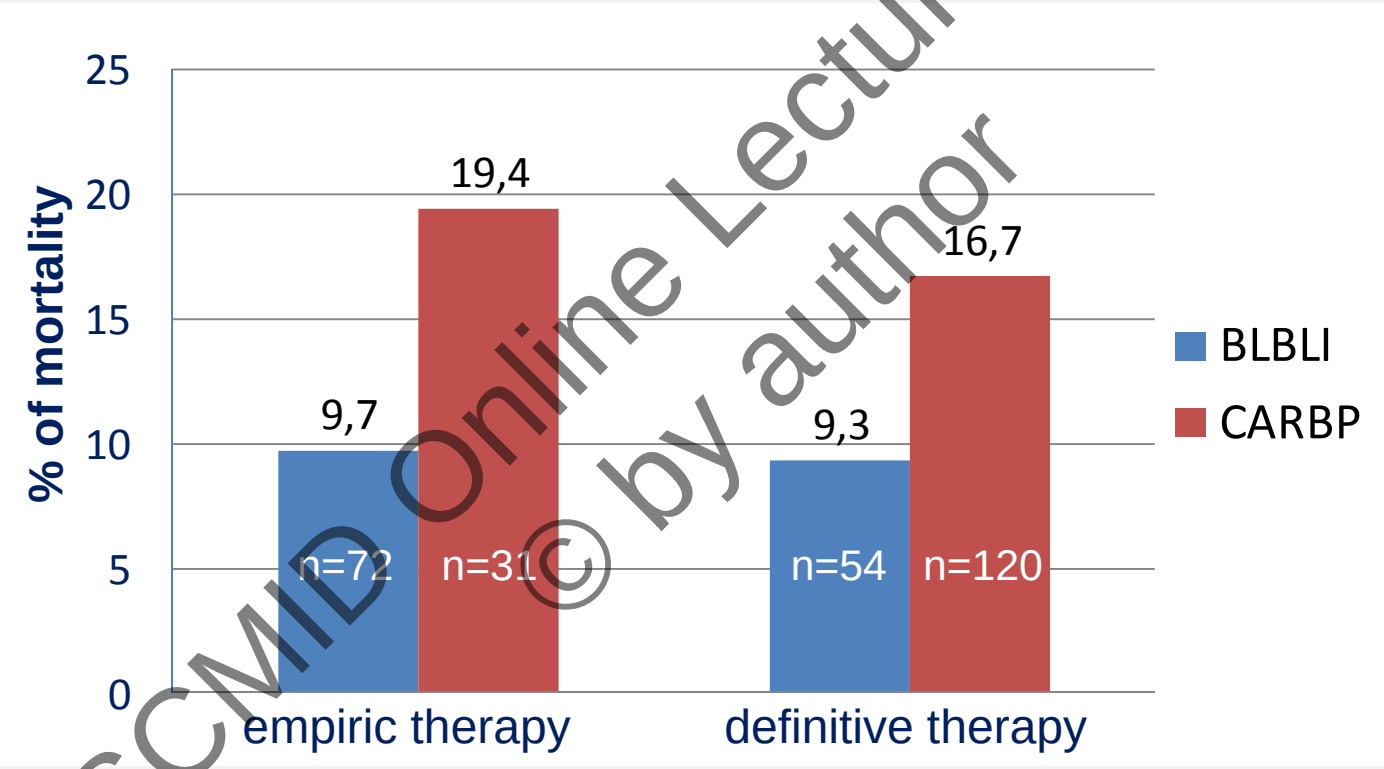





Gavin et al.Antimicrob Agents Chemother 2012; 50: 2244-7

# β-lactam - β-lactamase inhibitor combinations (BLBLI)

Mortality rates at day 30 in ESBL-*Escherichia coli* bacteriemic patients treated with BLBLI or carbapenems






Rodriguez-Baño et al.Clin Infect Dis 2012; 54: 167-174

# β-lactam - β-lactamase inhibitor combinations (BLBLI)

**Mortality rates** at day 30 in ESBL-*Escherichia coli* bacteriemic patients who received empirical terapy with an active BLBLI

| | Minimum Inhibitory Concentration, mg/L | | | | |
|---|---|---|---|---|---|
| Antimicrobial | ≤1 | 2 | 4 | 8 | 16 |
| Piperacillin-tazobactam | 0/10 | 0/8 | 1/4 | 2/6 | 1/7 |
| Amoxicillin-clavulanate | … | … | 1/12 | 2/25 | … |

[a] Data are expressed as No. of patients who died/No. of patients treated.

PTZ and AMC are suitable alternatives to carbapenems in ESBL-*E. coli* bacteriemic patients if active *in vitro* and would be particularly useful as definitive therapy

Rodriguez-Baño et al.Clin Infect Dis 2012; 54: 167-174

# EUCAST expert rules *v2:* major modifications

## β-lactams and *Haemophilus influenzae*

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

| Agents tested | Agents affected | Rule | Exceptions, scientific basis and comments |
|---|---|---|---|
| Ampicillin or amoxicillin (and β-lactamase detection) | Ampicillin, amoxicillin and piperacillin | IF β-lactamase positive THEN report as R to ampicillin, amoxicillin and piperacillin | Ampicillin is the class representative for amoxicillin<br>Resistance to ampicillin by production of β-lactamase may be misidentified by the disk diffusion technique.<br>Production of β-lactamase should be examined with a chromogenic test. |

# EUCAST expert rules *v2:* major modifications

## β-lactams and *Haemophilus influenzae*

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

| Agents tested | Agents affected | Rule | Exceptions, scientific basis and comments |
|---|---|---|---|
| Ampicillin or amoxicillin (and β-lactamase detection) | Ampicillin, amoxicillin, amoxicillin-c lavulanate, ampicillin-sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin and piperacillin-tazobactam. | IF BLNAR THEN report as R to ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, cefaclor, cefuroxime and cefuroxime axetil. | 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. |
| 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)

| Agents tested | Agents affected | Rule | Exceptions, scientific basis and comments |
|---|---|---|---|
| Amoxicillin-clavulanate (and β-lactamase detection) | Ampicillin- sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin and piperacillin-tazobactam. | IF BLPACR THEN report as R to ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, piperacillin, piperacillin-tazobactam. cefuroxime and cefuroxime axetil. | 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. |
| 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**

**Negative**

**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

**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)**

**R < 12 mm** → β-lactamase test

**Negative**

**Ampicillin-R (BLNAR)**

**Expert rule 10.2**

Report R to
Ampicillin, Amoxicillin
Amox/clav, Amp/sulb
Piperacillin, Piper/tazb
Cefaclor, Cefuroxime

**Positive**

**Ampicillin-R Amox/clav-S (BLP)**

**Expert rule 10.1**

Report R to:
Ampicillin
Amoxicillin
Piperacillin

**Ampicillin-R Amox/clav-R (BLPACR)**

**Expert rule 10.3**

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

# EUCAST expert rules *v2*

## Q3