# How to translate an antibiogram into a treatment ? Gram-negative bacteria

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# Major antimicrobial resistance problems in Gram-negative bacteria

- ***Enterobacteriaceae*** (*E. coli*, *Klebsiella*, *Enterobacter*)
  - ✓ Beta-lactams (3rd, 4th generation cephs)
    - Extended-spectrum Beta-lactamases (ESBL)
    - Cephalosporinases
  - ✓ Fluoroquinolones
    - Mutation in chromosomal genes (gyrA/B, Par C/E)

- ***Non-fermenters*** (*Pseudomonas*, *Acinetobacter*)
  - ✓ Beta-lactams (3rd, 4th generation cephs)
    - Cephalosporinases
    - ESBL
    - Carbapenemases
    - Altered permeability / Active efflux
  - ✓ Multi-drug resistance (β-lactams, aminoglycosides, fluoroquinolones)

# Extended-spectrum beta-lactamases (ESBL)

- Plasmid-mediated enzymes (TEM, SHV, CTX-M, OXA, VEB, PER,…)
- Mediate resistance to by hydrolysis of extended-spectrum cephalosporins (3$^{rd}$ and 4$^{th}$ generation) and monobactams (aztreonam)
- Do not inactivate carbapenems, cephamycins (cefoxitin), temocillin
- Activity inhibited by β-lactamases inhibitors (clavulanate, tazobactam)

# Characteristics of ESBLs

| Enzyme family | TEM | SHV | OXA | CTX-M |
|---|---|---|---|---|
| Total number in family | 135 | 54 | 57 | 34 |
| Number of ESBLs | 108 | 52 | 12 | 34 |
| Amino acids in enzyme | 286 | 292 | 266 | 290 |
| Nr of AA positions with substitutions | 37 | 32 | 19 | Sequences may differ by 20-25% |
| Maximum Nr of mutations | 6 | 7 | 9 | ND |
| Most common substitutions | E104K, R164S, R164H, M182T, E240K | L35Q, G238S, G238A | I10T, G20S, T110S, Y184F, E240G, S258S | ND |

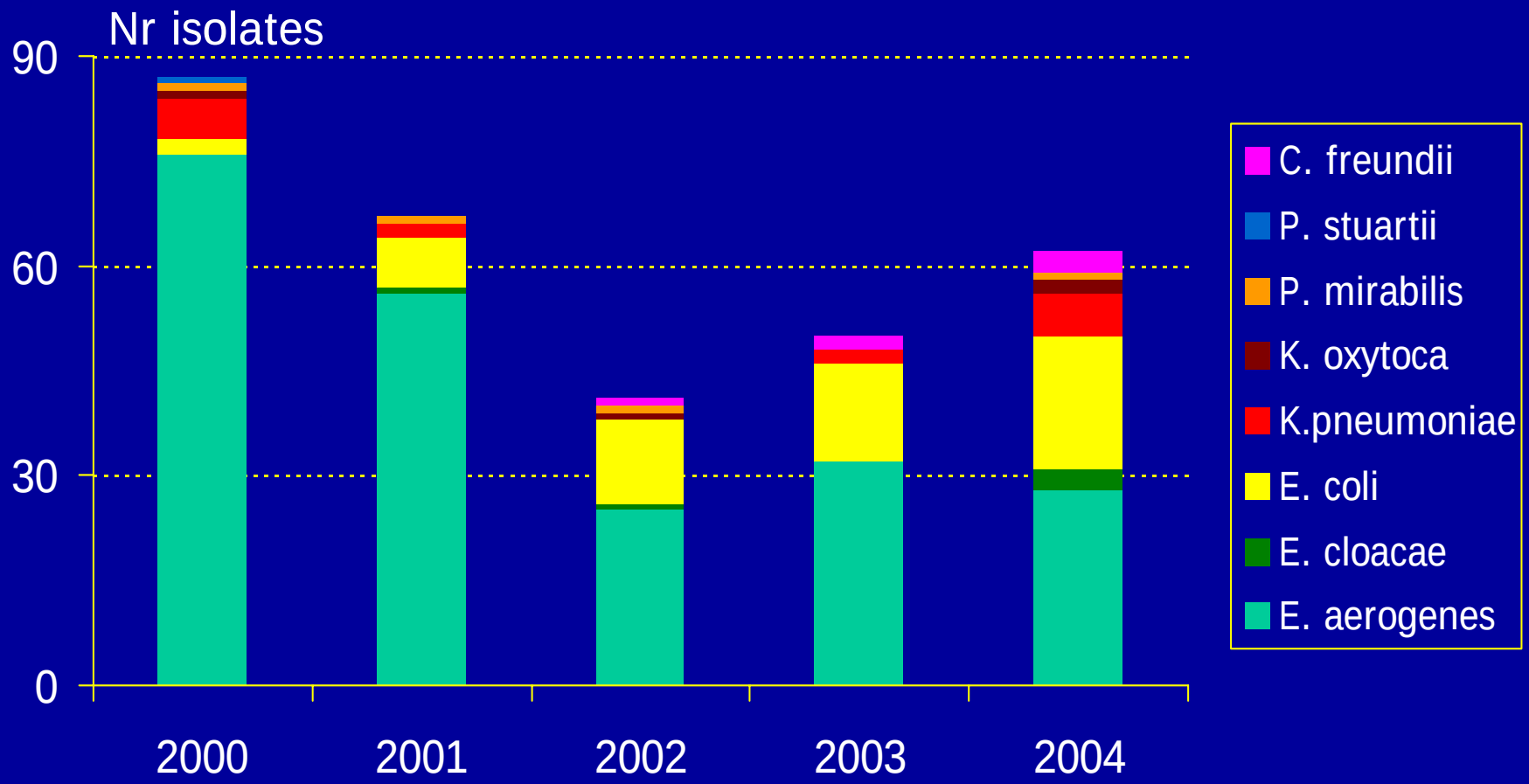
# Prevalence of ESBL Producing *Enterobacteriaceae* in Europe

| Country | % ESBL | | | | | |
|---|---|---|---|---|---|---|
| | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 |
| Russia | 24 | 34 | 42 | 47 | 22 | 30 |
| Poland | 37 | 23 | 21 | 40 | 33 | 37 |
| Turkey | - | - | 23 | 40 | 21 | 26 |
| Czech Republic | 5 | 8 | 8 | 6 | 14 | 10 |
| Italy | 40 | 10 | 15 | 9 | 11 | 7 |
| UK | 5 | 7 | 22 | 7 | 6 | 11 |
| Germany | 2 | 3 | 1 | 5 | 2 | 3 |
| Belgium | - | 6 | 5 | 8 | 5 | 8 |

MYSTIC data

# Distribution of ESBLs among *Enterobacteriaceae* species

UCL Mont-Godinne (2000-2004)



# ESBL-producing Gram-negative bacteria: Risk factors for infection and impact of resistance on outcome

- Matched case-control study (33 pts with infections due to ESBL isolates vs 66 controls with ESBL-negative isolates)
- Independent Risk factors: total prior antibiotic exposure (3rd gen cephs; OR: 16; $p<0.01$; cotrimoxazole; OR: 20; $p= 0.004$)
- Longer median duration of hospital stay (11 days vs 7 days) and higher total hospital charges (66,000 $ vs. 22,000 $) in cases versus controls
- Documentation of the spread of several closely-related clusters of E.coli/klebsiella ESBL + isolates

*Lautenbach, CID 2001*

# ESBL: detection problems

- Over 150 enzymes with variable spectrum and often low level resistance to extended-spectrum cephalosporins (cetriaxone, ceftazidime, cefepime...) and monobactams (aztreonam)
- Increasing number of species in which ESBL can be found
- MICs of ESBL-producing strains may be below susceptible breakpoints; (suspicion if MIC $\geq$ 2 µg/ml; NCCLS)
- Inhibition by clavulanate may be masked by concomitant production of AmpC enzymes (e.g. *Enterobacter* spp.)
- Chromosomal AmpC beta-lactamases may be mobilized on transferable plasmids to species in which ESBL are prevalent (e.g. *E. coli*, *Klebsiella* spp)

# NCCLS/CLSI MIC Interpretation

| Drug | Current NCCLS MIC Categories | | |
|---|---|---|---|
| | S | I | R |
| Aztreonam | 8 | 16 | 32 |
| Cefotaxime | 8 | 16-32 | 64 |
| Ceftriaxone | 8 | 16-32 | 64 |
| Cefepime | 8 | 16 | 32 |

M100-S15

# Methods for detection of ESBLs

**Strategy of the NCCLS** (USA)

- **Screening**

| disks: | (CPDOX, | CAZ, | CTX, | CTRX, | ATM) |
|---|---|---|---|---|---|
| (∅ mm) | | ≤ 17 | ≤ 22 | ≤ 27 | ≤ 25 | ≤ 27 |

MIC: ≥ 2 µg/ml (C3, C4, ATM); ≥ 8 µg/ml (CPDOX)

- **Confirmation**

- Combination disks (CAZ, CTX + CA): ≥ 5 mm vs CAZ / CTX alone
- MIC CAZ / CTX + CA: ≤ 8 vs MIC of CAZ / CTX alone

ESBL E-test strips or combination disks (Oxoid)
(sensitivity CAZ/ CTX: 90-95%)

# Screening Tests for ESBLs in *K. pneumoniae, K oxytoca*, *E. coli* and *P. mirabilis*

- Screen: MIC $\geq$ 2 µg/ml for ceftazidime, aztreonam, cefotaxime or ceftriaxone or $\geq$ 8 µg/ml for cefpodoxime (use of more than one drug will improve sensitivity of detection)
- Confirm: 8-fold or greater reduction in MIC in combination with clavulanic acid

NCCLS Document M100-S15, Jan 2005

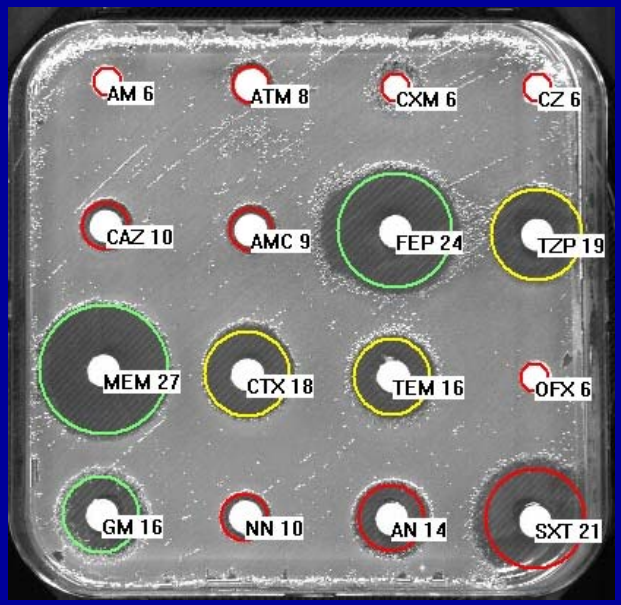
# Interpretation of antimicrobial susceptibility for ESBLs producing organisms

- **NCCLS**
  - Guidelines only for *E. coli*, *Klebsiella* spp., *P. mirabilis*
  - Interpret as resistant all cephalosporins including C3, C4 and aztreonam
- **CA-SFM**
  - Guidelines for all *Enterobacteriaceae* species
  - Interpret S $\rightarrow$ I, I $\rightarrow$ R all cephalosporins including C3, C4 and aztreonam

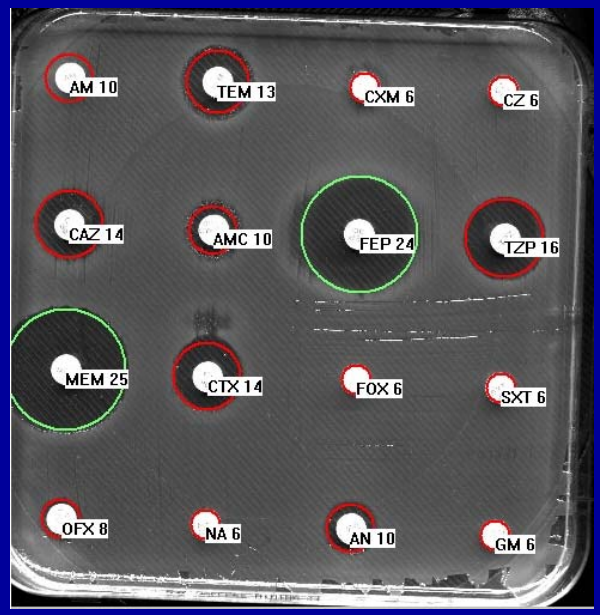
*No interpretative guidelines for β-lactam/ β-lactamase inhibitor combinations (e.g.: Amox/Clav., Pip/Tazo.)*

# Expression of various types of ESBLs in different bacterial species

*E. aerogenes*
*SHV-4* (+ AmpC)




*E. coli*
TEM-3 (+ AmpC)




*C. freundii*
TEM-24

# Inoculum effect in tests of various β-lactams with ESBL-producing *E. coli*

| Inoculum (CFU/ml) and antibiotic | Range | $MIC_{50}$ (µg/ml) | $MIC_{90}$ (µg/ml) | % susceptible |
|---|---|---|---|---|
| $10^5$ | | | | |
| Meropenem | ≤0.015-0.06 | ≤0.015 | 0.03 | 100 |
| Cefotaxime | 0.25-512 | 2 | 64 | 79 |
| Ceftazidime | 1-1024 | 32 | 256 | 32 |
| Cefepime | 0.25-128 | 2 | 128 | 79 |
| Pip-Tazo | 1-32 | 2 | 8 | 95 |
| $10^7$ | | | | |
| Meropenem | 0.03-0.05 | 0.06 | 0.12 | 100 |
| Cefotaxime | 2-1024 | 256 | >1024 | 21 |
| Ceftazidime | 4->1024 | >1024 | >1024 | 5 |
| Cefepime | 4->128 | >128 | >128 | 5 |
| Pip-Tazo | 1-1024 | 8 | 1024 | 16 |

* Increase of MIC by ≥ 8 at 100-fold higher inocula

*Thomson, AAC 2001; 45: 3548*

# Importance of ESBL production on choice of antibiotic therapy and clinical outcome

- Multicenter prospective study of *K. pneumoniae* bacteremia (454 episodes, 12 centres US/Europe)
- 85 episodes (18%) due to ESBL-producing isolates
- Failure to use antibiotic active against ESBL-producing isolates associated with high mortality rate at day 14 (>60% vs 14% when AB active in vitro )
- Use of carbapenem within 5 day of bacteremia associated with lower mortality at day 14 than with other ABs active in vitro:
  - Imipenem/meropenem (3%)
  - Quinolones monotherapy: (36%)
  - Cephalosporin or β-lactam/ β-lactamases inhibitors: (44%)

→ ***Need for appropriate antibiotic choice in severe infections caused by ESBL-producing organisms***

*Paterson, Ann. Intern. Med., 2004*

# Outcome of treatment with a broad-spectrum cephalosporin in severe infections according to the MIC value of the ESBL-producing isolate

| MIC (µg/ml) | Treatment failure at 72h | 30-day mortality |
|---|---|---|
| ≤ 1 | 0/2 (0) | 0/2 (0) |
| 2 | 1/4 (25) | 1/4 (25) |
| 4 | NC | NC |
| 8 | 2/2 (100) | 1/2 (50) |
| 16 | 4/6 (66,7) | 3/6 (50) |
| 32 | 8/11 (72,7) | 3/11 (27,3) |

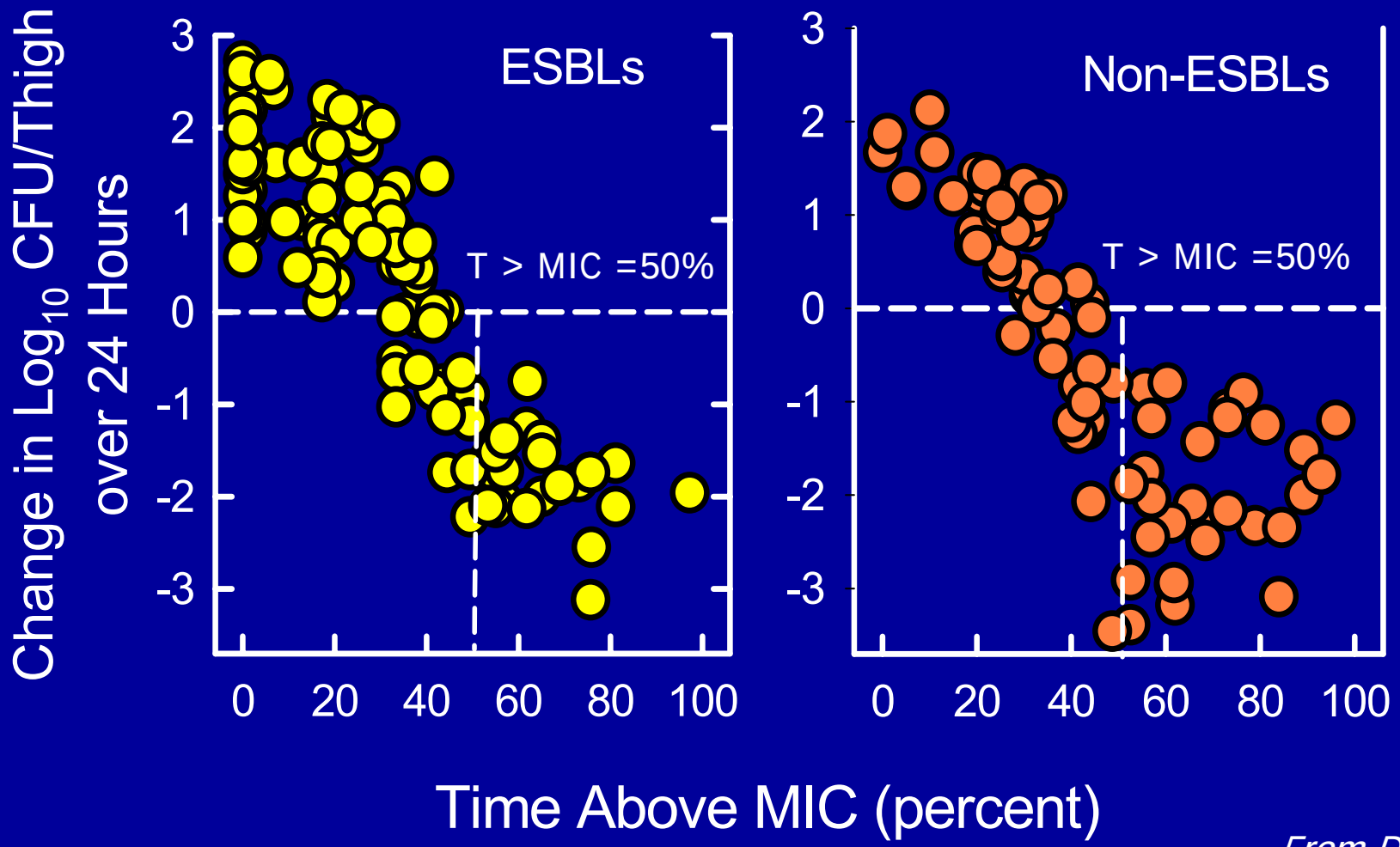
*Kang, AAC 2004*

# Clinical outcome in 42 Patients with ESBL-producing Klebsiella/E. coli bacteremia and treated with cephalosporin monotherapy

| Outcome | MIC ≤1 µg/L | MIC 2 µg/L | MIC 4 µg/L | MIC 8 µg/L |
|---|---|---|---|---|
| Success | 13 (81%) | 4 (67%) | 3 (27%) | 1 (11%) |
| Failure | 3 (19%) | 2 (33%) | 8 (73%) | 8 (89%) |

Paterson et al J Clin Micro 39:2206, 2001; Kim et al AAC 46:1481, 2002; Wong-Beringer et al Clin Infect Dis 34:135, 2002; Kang et al AAC 46: 4574; 2004; Bhavani et al 44rd ICAAC, Abstract K-1588, 2004

# Activity of 4 Cephalosporins against Various *Enterobacteriaceae* with and without ESBLs in Murine Thigh-Infection Model




*From D Andes*

# Clinical features and outcome of bacteremia caused by AmpC-type resistant Gram-negative isolates

- 389 episodes of Klebsiella pneumoniae bacteremia (1998-2002); 65 (17%) due to AmpC + or ESBL + 3rd G ceph-resistant isolates
- No difference in clinical severity and risk factors between AmpC group and ESBL group at time of presentation
- No difference in initial treatment failure at day 3 (52%) and mortality rate at day 7 (15%) and day 30 (29%) between the two groups (AmpC and ESBL)
- Mortality rate in 5/8 (62%) pts who received 3rd G ceph as definitive therapy (all MICs $\geq$ 32 µg/ml) and in 1/11 (9%) pts who received imipenem or ciprofloxacin treatment

*BN Kim, Int J Animicrob Agents 2003; 22: 106*

# Cefepime for the treatment of infections due to ESBL-producing *Enterobacter aerogenes*

- Treatment of infections caused by ESBL-producing *E. aerogenes* in ICU patients (n=44)
- 60% pneumonia; 2 BSI (cefepime) – 5 BSI (meropenem)
- Cefepime 3 x 2 g (n= 21); Imi/Meropenem 3 x 1 g (n=23)
- No statistical difference between groups in mortality, nor in clinical / bacteriological outcome
- Shorter duration of treatment in cefepime (8.5 d) vs meropenem group (11,4 d)
- No difference in outcome between cefepime alone vs. Cefepime + amikacin

*Goethaert et al. ICAAC 2003. Poster K-71*

# PK/PD of Cefepime and Pipe-Tazo against *E. coli / Klebsiella* spp. Strains producing ESBL

| Regimen | *P for achieving* T>MIC target measure | | | | |
|---|---|---|---|---|---|
| | 30% | 40% | 50% | 60% | 70% |
| *E.coli* | | | | | |
| Pipe-tazo 3.375g/4h | 96 | 92 | 90 | 86 | 77 |
| Pipe-tazo 3.375g/6h | 91 | 86 | 73 | 50 | 28 |
| Cefepime 2 g/12h | 100 | 100 | 100 | 100 | 99 |
| Cefepime 1 g/12h | 100 | 99 | 99 | 98 | 96 |
| *K. pneumoniae* | | | | | |
| Pipe-tazo 3.375g/4h | 77 | 72 | 65 | 57 | 48 |
| Pipe-tazo 3.375g/6h | 69 | 57 | 43 | 29 | 16 |
| Cefepime 2 g/12h | 100 | 100 | 100 | 98 | 96 |
| Cefepime 1 g/12h | 99 | 96 | 95 | 94 | 93 |

*Ambrose, AAC 2003; 47: 1643*

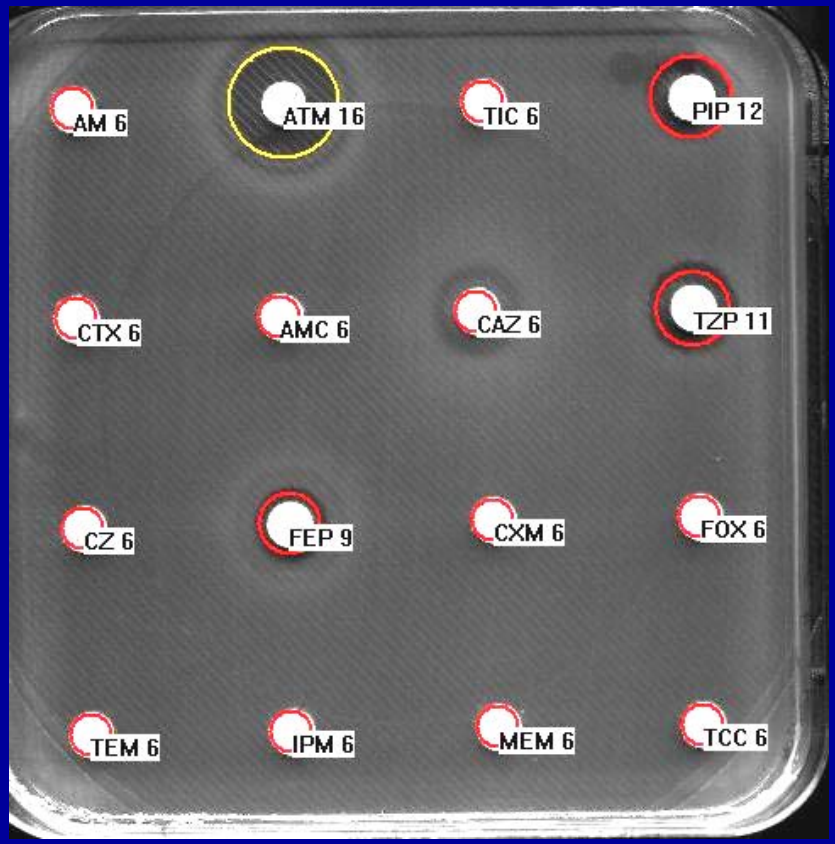
# In vitro activity of temocillin against ESBL-producing *Enterobacteriacae* isolates

| Species (n) | Temocillin % Susceptibility | MIC50 (µg/ml) | MIC90 (µg/ml) | ESBL type enzymes |
|---|---|---|---|---|
| *Enterobacter aerogenes* (172) | 93 | 4 | 16 | 82% TEM |
| *Enterobacter cloacae* (104) | 99 | 2 | 8 | 90% SHV-12 + TOHO2 |
| *Escherichia coli* (164) | 92 | 8 | 16 | 43% TEM<br>37% TEM +CTX-M |
| *Klebsiella pneumoniae* (58) | 95 | 2 | 16 | 47% TEM+ SHV + CTX-M |
| All species (533) | 94 | 4 | 16 | |

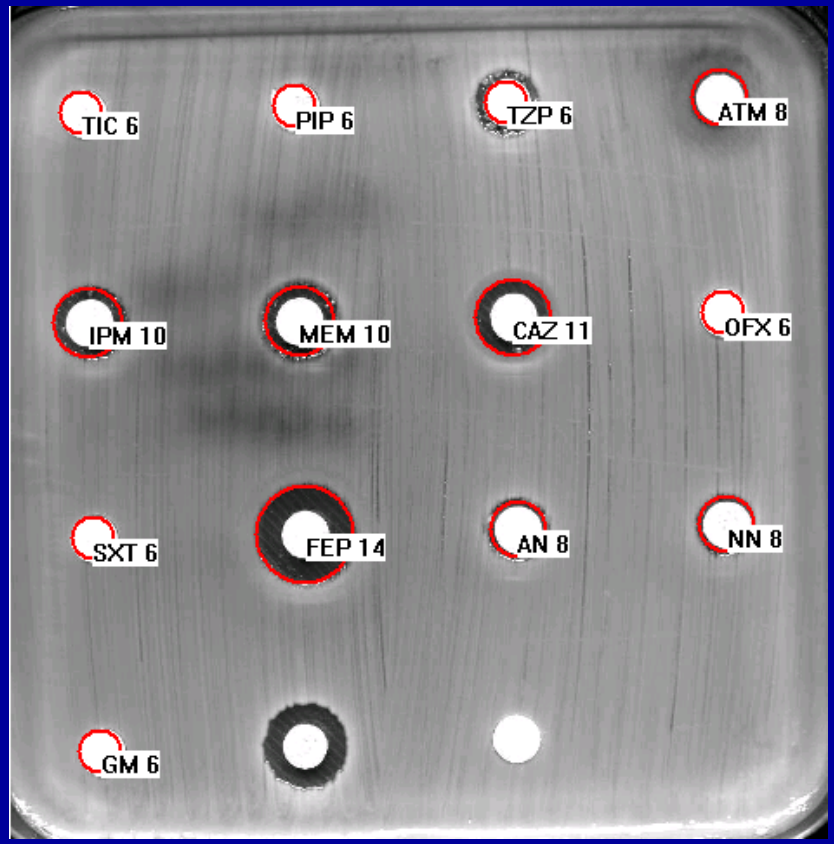
Susceptible MIC ≤ 16 µg/ml

*Rodriguez-Villalobos, 15th ECCMID 2005, Abstract P1221*

# Difficult-to-treat organisms
# Gram-negative non-fermenters




*P. aeruginosa*




*A. baumannii*

# Difficult-to-treat organisms
# Gram-negative non-fermenters

- Intrinsic resistance to several classes of agents, susceptibility profile poorly predictible
- High propensity to develop resistance in vivo during therapy (acquired or mutational resistance mechanisms)
- High level of resistance and multi-drug resistance through addition of several resistance mechanisms
  (poor probability of critical PK/PD attainment)
- Few existing therapeutic options, no new agents in development

# Antimicrobial susceptibility of *P. aeruginosa* isolates in Belgian hospitals

**Table 1.** Results of susceptibility testing

| | Susceptible[a] | Intermediate[a] | Resistant[a] | $MIC_{50}$ (mg/L) | $MIC_{90}$ (mg/L) |
|---|---|---|---|---|---|
| Gentamicin | 67 | 9.5 | 23.5 | 4 | 256 |
| Tobramycin | 79.5 | 1 | 19.5 | 1 | 128 |
| Amikacin | 85 | 4.5 | 10.5 | 8 | 64 |
| Isepamicin | 81 | 7 | 12 | 8 | 64 |
| Ofloxacin | 49.75 | 12.5 | 37.75 | 4 | 128 |
| Levofloxacin | 61.5 | 11 | 27.5 | 2 | 64 |
| Ciprofloxacin | 71 | 5 | 24 | 0.5 | 32 |
| Piperacillin | 76 | – | 24 | 16 | >256 |
| Piperacillin/tazobactam | 83 | – | 17.5 | 16 | 256 |
| Ticarcillin/clavulanic acid | 63 | – | 37 | 64 | 256 |
| Aztreonam | 17.75 | 26.75 | 55.5 | 32 | 256 |
| Ceftazidime | 59 | 12.5 | 28.5 | 8 | 64 |
| Cefepime | 50.5 | 20 | 29.5 | 8 | 64 |
| Meropenem | 81.5 | 9 | 9.5 | 2 | 8 |

[a]Percentage of all isolates.

*Van Eldere, JAC 2003; 51: 347-52*

# Emergence of antimicrobial resistance of *P. aeruginosa* in relation to previous drug exposure

271 pts; (Follow-up 3810 j): Resistance 10,2% (7.4/1000 pts.day)

| Drug administred | Event (N° / total Rx) | OR-Res | OR-Res same AB | P |
|---|---|---|---|---|
| Ceftazidime | 10/125 | 0.7 | 0.8 | .7 |
| Ciprofloxacin | 12/98 | 0.8 | 9.2 | .04 |
| Imipenem | 11/37 | 2.8 | 44.1 | .001 |
| Piperacillin | 9/91 | 1.7 | 5.2 | .01 |

Carmeli et al., AAC 1999

# Carbapenemases

- **Zinc β-lactamases** (Classe B): IMP-1/17, VIM-1/10
  - *P. aeruginosa, Acinetobacter, Enterobacteriaceae….*
  - Mobile genes (Integrons-transposons-plasmids)
  - Broad-spectrum, high level-resistance to all β-lactams (except aztreonam)
  - South-East Asia, Italy, Greece, France
  - No clear relationship with previous exposure to carbapenems
- **Other β-lactamases** (Classe D, A): OXA-23,24,25,26,40, 58… SME-1, NMC-A, KPC-1/2
  - *Acinetobacter spp. E. cloacae*, *Serratia*, *Klebsiella*
  - Variable level of resistance to carbapenems (Imi $\geq$ Mero)
  - Wide diffusion of OXA-type carbapenemases in Acinetobacters in many countries

# Detection of carbapenemases

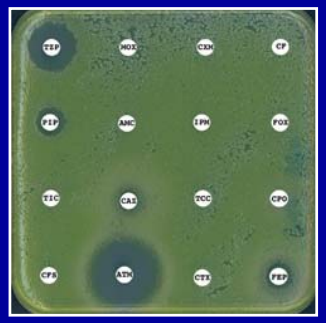
**Variable phenotypic expression**

Resistance to all beta-lactams, variable resistance levels to imi/merop
Multi-drug resistant (aminoglycosides, cotrimoxazole, tetra, quinolones)

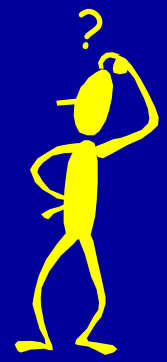
Metallo-beta-lactamase: MIC > 256 µg/ml (Imi/mero)
MIC > 256 µg/ml (ceftaz, cefep)

Synergy between imipenem (or ceftazidime) and EDTA (or 2-MPA)
False-positive and false-negative results
Metallo-B-lactamases (VIM, IMP): S to aztreonam

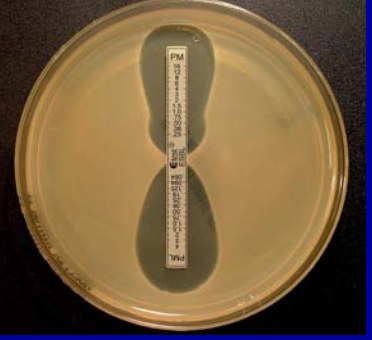
Other carbapenemases (Class D) difficult to detect by phenotypic test:
Increase of MIC to Imi > mero (2-8 µg/ml)



# What to do ?



- Test and report only agents for which the species constitutes a potential target for therapy
- Do not extrapolate susceptibility results from one agent to another within a class (e.g. imipenem, meropenem)
- Quantitative MIC testing required in severe infections (bacteremia, ICU patients)
- Repeat testing during therapy (every 3-7 d) to screen for emergence of resistance during therapy



# What to do ?

- Consider in vitro synergy tests between different drugs classes (checkerboard, killing curves) (e.g. cystic fibrosis)
  - Beta-lactams, aminoglycosides, quinolones
- Consider testing of old drugs (e.g. colistin) or agents that might potentially be useful in combination therapy
  - Acinetobacter: sulbactam, tigecycline
  - Pseudomonas: rifampin, fosfomycin

# In vitro susceptibility testing of quinolones against Gram-negative organisms

- Species-related clinical MIC breakpoint of fluoroquinolones dependent on MIC distribution in a species (EUCAST)
  - Enterobacteriaceae/Pseudomonas: Ciprofloxacin S ≤ 0.5 µg/ml; R >1 µg/ml
  - N. gonorrhoea/N. meningitidis: S ≤ 0.03 µg/ml; R > 0.06 µg/ml

- Clinical or PK/PD related breakpoints (EUCAST – NCCLS)

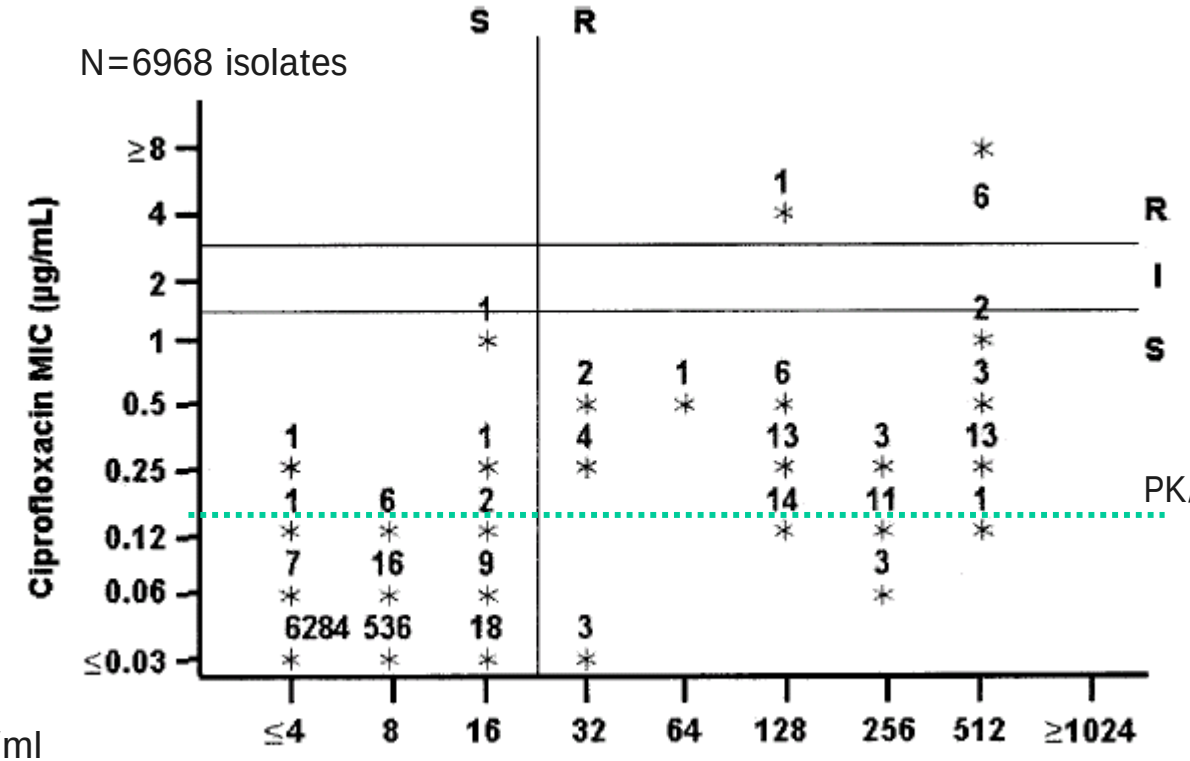
  (Test extra-intestinal salmonella isolates for resistance to nalidixic acid; Inform clinician that Nalidixic ac.-R isolates might not respond to fluoroquinolone Rx)

- Screen for low-level resistance in *Enterobacteriaceae* by testing least active compounds (norfloxacin)

  (CA-SFM: Activity of fluoroquinolones should be individually tested for Norfloxacin-**I** or –R isolates)

# MIC scatterplots for nalidixic acid versus ciprofloxacin for non-Typhi salmonellae

(NCCLS, 1996-2000)




S ≤ 1 µg/ml
R ≥ 4 µg/ml

$C_{max}$ = 2,5 µg/ml
$AUC_{24h}$ = 23 h.µg/ml

S ≤ 16 µg/ml
R ≥ 32 µg/ml

*Crump, CID 2003*

# In vitro susceptibility testing of quinolones against Gram-negative organisms

- Do not report results for species that are poor target for therapy with the drug or when there is a lack of data
  - e.g. Moxifloxacin and Pseudomonas
  - E.g. Levofloxacin and Gram-negative anaerobes
- Do only report results with most active compounds within a class
  - e.g. ciprofloxacin and Pseudomonas
- Quantitative results (MIC/zone sizes) in case of severe infections (bacteremia, ICU patient) and/or in difficult-to-treat organisms