

# **Antimicrobial susceptibility testing: current and emerging problems and application of expert rules**

## **Gram-negative rods and resistance to non- $\beta$ -lactam drugs**

**EUCAST EDUCATIONAL WORKSHOP**  
*19th ECCMID Helsinki, Finland, 2009*

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EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

# Non- $\beta$ -lactam drugs and EUCAST Expert Rules

## Gram negative rods

- Non- $\beta$ -lactam antimicrobials are rarely included as a first option in clinical guidelines
- Less information available on ...
  - correlation of MIC values and clinical outcomes
  - treatment failure descriptions
- No new drugs in some of the groups (i.e. aminoglycosides)
- More difficult to infer non- $\beta$ -lactam resistance mechanisms
  - more experience with  $\beta$ -lactams and “interpretive reading”
  - experts prefer  $\beta$ -lactams than non- $\beta$ -lactams

*but ...*

# Non- $\beta$ -lactam drugs and EUCAST Expert Rules

## Gram negative rods

- Dramatic increase of resistance in some groups (fluoroquinolones)
- Increasing information of new resistance mechanisms due to the application of molecular techniques
  - transferable genetic determinants
  - low level expressed resistance mechanism
- Increasing information of Pk/Pd data
- Revitalization of “old” antimicrobials (colistin, fosfomycin, ...)
- Introduction of new antimicrobials (or variants of previous ones)
  - glycylicyclines (tigecycline)
- Different patterns of use:
  - in hospitals and in the community
  - geographic areas

# What do we have in the EUCAST Expert Rules for Gram-negative rods and resistance to non- $\beta$ -lactam drugs?

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Intrinsic resistances (Table 1-3)

Exceptional phenotypes (Table 5)

Interpretive rules for aminoglycosides (Table 12)

Interpretive rules for fluoroquinolones (Table 13)

# Gram-negative rods and resistance to non-β-lactam drugs

## Intrinsic resistances (Table 1)

| Rule no. | Organisms                          | Aminoglycosides   | Tetracyclines<br>tigecycline | Polymyxin B<br>Colistin | Nitrofurantoin |
|----------|------------------------------------|-------------------|------------------------------|-------------------------|----------------|
| 1.8      | <i>Morganella morganii</i>         |                   | R                            | R                       | R              |
| 1.9      | <i>Proteus mirabilis</i>           |                   | R                            | R                       | R              |
| 1.10     | <i>Proteus vulgaris</i>            |                   | R                            | R                       | R              |
| 1.11     | <i>Proteus penneri</i>             |                   | R                            | R                       | R              |
| 1.12     | <i>Providencia rettgeri</i>        | R <sup>2</sup>    |                              | R                       | R              |
| 1.13     | <i>Providencia stuartii</i>        | R <sup>2</sup>    |                              | R                       | R              |
| 1.14     | <i>Serratia marcescens</i>         | Note <sup>3</sup> |                              | R                       |                |
| 1.15     | <i>Yersinia enterocolitica</i>     |                   |                              |                         |                |
| 1.16     | <i>Yersinia pseudotuberculosis</i> |                   |                              | R                       |                |

<sup>2</sup> All *Providencia* spp. produce a chromosomal AAC(2')-Ia enzyme. *Providencia* spp. should be considered R to all aminoglycosides except amikacin and streptomycin. Some isolates express the enzyme poorly and can appear S to netilmicin *in vitro*, but should be reported as R as mutation can result in overproduction of this enzyme

<sup>3</sup> All *S. marcescens* produce a chromosomal AAC(6')-Ic enzyme that may affect moderate the activity of all aminoglycosides except streptomycin and gentamicin

# Gram-negative rods and resistance to non-β-lactam drugs

## Intrinsic resistances (Table 2)

| Rule no. | Organisms  | Ciprofloxacin | Chloramphenicol | Aminoglycosides   | Trimethoprim   | Fosfomycin | Tetracyclines<br>Tigecycline | Polymyxin B<br>Colistin |
|----------|--|---------------|-----------------|-------------------|----------------|------------|------------------------------|-------------------------|
| 2.1      | <i>Acinetobacter baumannii</i> ,<br><i>Acinetobacter calcoaceticus</i> |               |                 |                   | R              | R          |                              |                         |
| 2.3      | <i>Burkholderia cepacia</i> complex <sup>2</sup>                       | R             | R               | R <sup>3</sup>    | R              | R          |                              | R                       |
| 2.4      | <i>Chryseobacterium meningosepticum</i>                                |               |                 |                   |                |            |                              | R                       |
| 2.6      | <i>Pseudomonas aeruginosa</i>  |               | R               | Note <sup>4</sup> | R <sup>5</sup> |            | R                            |                         |
| 2.7      | <i>Stenotrophomonas maltophilia</i>                                    |               |                 | R <sup>3</sup>    | R <sup>7</sup> | R          |                              |                         |

<sup>3</sup> *B. cepacia* and *S. maltophilia* are intrinsically R to all aminoglycosides. Intrinsic resistance is attributed to poor permeability and putative efflux. In addition, most *S. maltophilia* produce AAC(6')Iz enzyme. On agar plates, resistance to aminoglycosides is more reliably detected after incubation at 30°C or ambient temp. than at 35-37°C.

<sup>4</sup> *P. aeruginosa* is intrinsically R to kanamycin and neomycin due to low level APH(3')-IIb activity.

<sup>5</sup> *P. aeruginosa* typically is R to trimethoprim and moderately S to sulphonamides. Although it may appear S in vitro to co-trimoxazole, it should be considered R.

<sup>7</sup> *S. maltophilia* typically is susceptible to co-trimoxazole, but resistant to trimethoprim alone.

# Tetracyclines and glycylyclines: Mechanisms of resistance

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- Tetracycline resistance widely distributed in Gram-negatives
  - high use in humans (70s), still in animals and agriculture
- Tetracycline resistance genes associated with mobile elements
- Different resistance mechanism with different epidemiology
  - efflux pumps
  - inactivation
  - ribosomal protection
  - unknown function
- Do not equally affect all tetracyclines
  - tetracycline > doxycycline > minocycline
- Glycylyclines (tigecycline) only affected by certain efflux pumps

Speer et al. Clin Microbiol Rev 1992; 5:387-99

Chopra. Drug Resist Updat 2002; 5:119-25

Livermore. J Antimicrob Chemoter 2005; 56:611-4

Shlaes. Curr Opin Investig Drugs 2006; 7:167-71

# Tetracyclines and gycylcyclines: Mechanisms of resistance

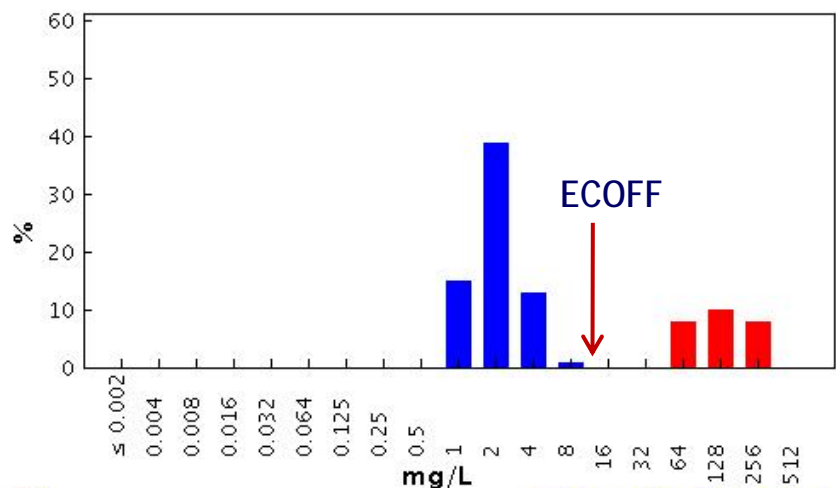
| Efflux                             | Ribosomal protection     | Enzymatic      | Unknown        |
|------------------------------------|--------------------------|----------------|----------------|
| <i>tet</i> (A), (B), (C), (D), (E) | <i>tet</i> (M)           | <i>tet</i> (X) | <i>tet</i> (U) |
| <i>tet</i> (G), (H), (I), (J)      | <i>tet</i> (O)           |                | <i>otr</i> (C) |
| <i>tet</i> (K), (L)                | <i>tet</i> (O), (S), (T) |                |                |
| <i>tet</i> (V)                     | <i>tet</i> (W)           |                |                |
| <i>tet</i> (Y), (Z)                | <i>tei</i>               |                |                |
| <i>tcr3</i>                        | <i>otr</i> (A)           |                |                |
| <i>tet</i> (30), (31)              | <i>tetP</i> (B)          |                |                |
| <i>otr</i> (B)                     |                          |                |                |
| <i>tetP</i> (A)                    |                          |                |                |

do not affect tigecycline

### Tetracycline / *Escherichia coli*

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution

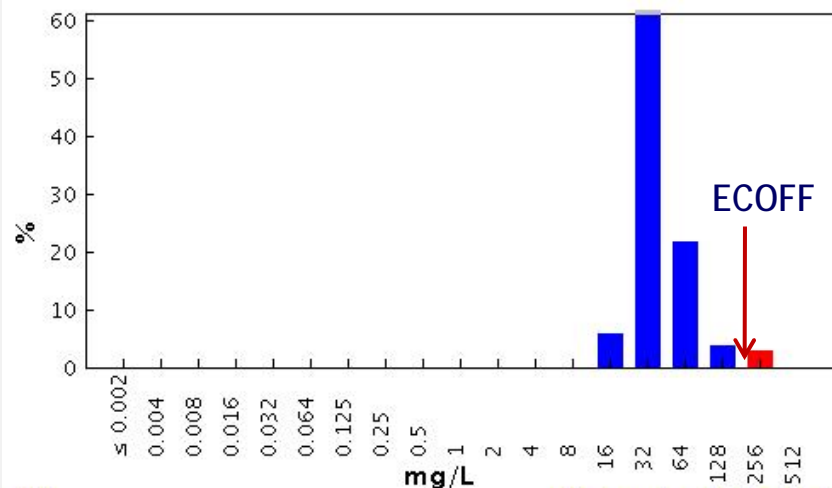


MIC Epidemiological cut-off: WT ≤ 8 mg/L  
 9252 observations (24 data sources)  
 Clinical breakpoints: Inappropriate

### Tetracycline / *Proteus mirabilis*

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution

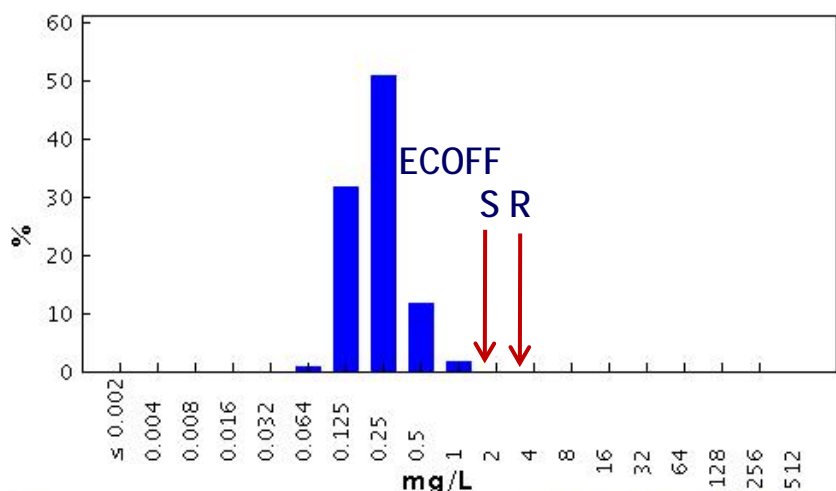


MIC Epidemiological cut-off: WT ≤ 128 mg/L  
 566 observations (4 data sources)  
 Clinical breakpoints: Inappropriate

### Tigecycline / *Escherichia coli*

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution

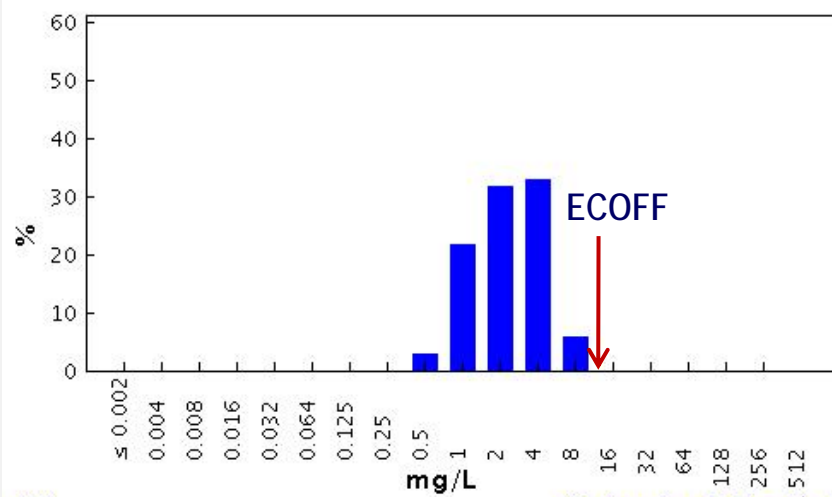


MIC Epidemiological cut-off: WT ≤ 1 mg/L  
 2608 observations (14 data sources)  
 Clinical breakpoints: S ≤ 1 mg/L, R > 2 mg/L

### Tigecycline / *Proteus mirabilis*

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution



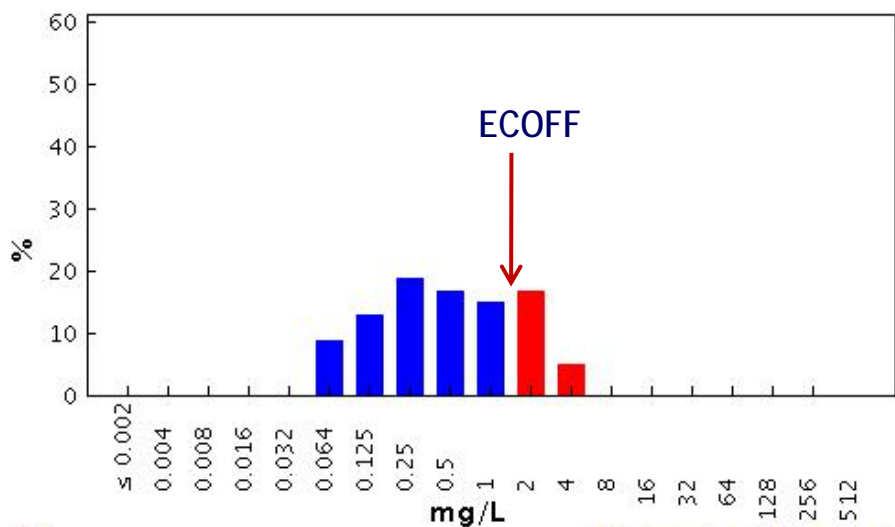
MIC Epidemiological cut-off: WT ≤ 8 mg/L  
 722 observations (8 data sources)  
 Clinical breakpoints: Inappropriate

# Gram-negative rods and resistance to non-β-lactam drugs

## Tigecycline

### Tigecycline / *Acinetobacter baumannii*

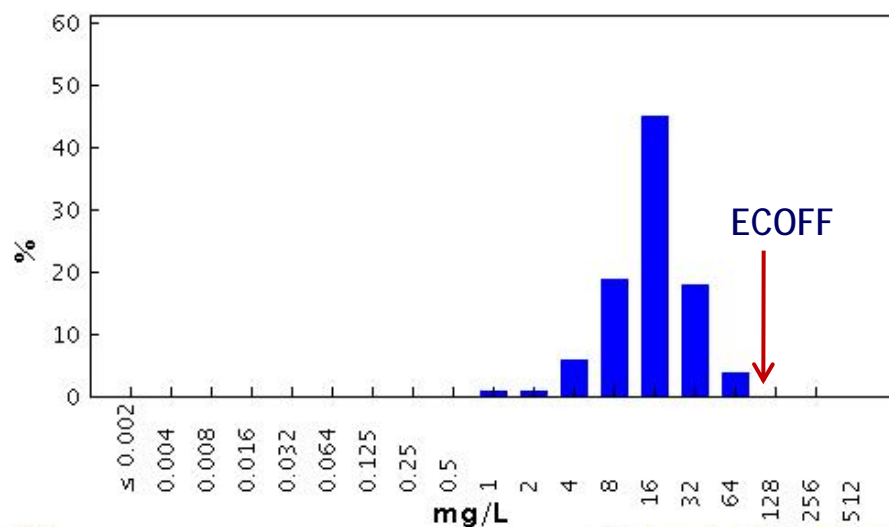
Antimicrobial wild type distributions of microorganisms - reference database  
EUCAST MIC Distribution



MIC  
Epidemiological cut-off: WT ≤ 1 mg/L  
190 observations (6 data sources)  
Clinical breakpoints: IE

### Tigecycline / *Pseudomonas aeruginosa*

Antimicrobial wild type distributions of microorganisms - reference database  
EUCAST MIC Distribution



MIC  
Epidemiological cut-off: WT ≤ 64 mg/L  
682 observations (7 data sources)  
Clinical breakpoints: Inappropriate

# Tetracyclines and glycylyclines: Pharmacokinetics

|                            | Tetracycline | Tigecycline |           |
|----------------------------|--------------|-------------|-----------|
|                            | 500 mg oral  | 100 mg iv   | 50 mg iv  |
| Cmax (mg/L)                |              |             |           |
| 30 min (IV)                | -            | 1.4±0.3     | 0.9±0.2   |
| 60 min (IV)                | -            | 0.9±0.3     | 0.6±0.1   |
| 2 h (oral)                 | 1.5-5        | -           | -         |
| T1/2 (h)                   | 6-12         | 27.1±14.3   | 42.4±35.3 |
| AUC <sub>∞</sub> (mg/L.h)  | -            | 5.2±1.9     | NA        |
| Vd (L)                     | 108          | 568±244     | 639±307   |
| Total body clearance (L/h) |              | 21.8±8.9    | 23.8±7.8  |

Sabundayo & Standiford. Antimicrobial Agents, 2005  
 Sun et al. Antimicrob Agents Chemother 2009; 49:1629  
 Meagher et al Clin Infect Dis 2005; 41 (Suppl 5):334  
 Muralidhan et al. Antimicrob Agents Chemother 2005; 49:220

# Tetracyclines and glycylyclines: Mechanisms of resistance

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*Proteae*: resistance due to hyperexpression of chromosomally encoded AcrAB efflux pump

| Strain: <i>Proteus mirabilis</i> | AcrA hyper-expression | Comple-mentation | Tn-Insertion | MIC (mg/L) |      |
|----------------------------------|-----------------------|------------------|--------------|------------|------|
|                                  |                       |                  |              | MIN        | TGC  |
| Wild type                        | +                     | -                | -            | 32         | 4    |
| Laboratory derived               | ++                    | +                | -            | >64        | 16   |
| Laboratory derived               | -                     | -                | +            | 1          | 0.25 |
| Laboratory derived               | ++                    | +                | +            | >64        | 16   |

MIN: minocycline; TGC: tigecycline

# Tetracyclines and glycylyclines: Mechanisms of resistance

*P. aeruginosa*: resistance due to chromosomally encoded MexXY-OprM efflux pump

| Deletions         | MIC (mg/L) |     |        |       |      |      |     |
|-------------------|------------|-----|--------|-------|------|------|-----|
|                   | TMP        | CHL | GEN    | CIP   | CARB | TET  | TGC |
| Wild type         | 64         | 250 | 1      | 0.06  | 160  | 16   | 8   |
| <i>mexB</i>       | 16         | ≤8  | 2      | 0.06  | 20   | -    | 8   |
| <i>mexXY</i>      | 125        | 125 | ≤0.125 | 0.06  | 160  | 4    | 0.5 |
| <i>mexB/mexXY</i> | 4          | 125 | ≤0.125 | ≤0.03 | 1.25 | 0.5  | 0.5 |
| <i>mexAB/oprM</i> | 4          | 32  | ≤0.125 | ≤0.03 | 1.25 | 0,25 | 0.5 |

TMP: trimethoprim; CHL: chloramphenicol; CIP: ciprofloxacin; CARB: carbenicillin; TET: tetracycline; TGC: tigecycline

# Expert rules for quinolones & Enterobacteriaceae

| Rule No. | Organism                  | Agent          | Rule  | Exceptions | Scientific basis   | Grade* | References  |
|----------|---------------------------|----------------|---|------------|--|--------|---|
| 13.6     | <i>Enterobacteriaceae</i> | Ciprofloxacin  | If resistant to ciprofloxacin, report as resistant to all fluoroquinolones  |            | Acquisition of at least two target mutations in <i>gyrA</i> plus <i>parC</i> or <i>gyrA</i>  | B      | Komp Lindgren P <i>et al.</i> , 2003  |
| 13.7     | <i>Salmonella</i> spp.    | Nalidixic acid | If resistant to nalidixic acid, report as resistant to all fluoroquinolones |            | Evidence for clinical failure of fluoroquinolones in case of resistance to nalidixic ac. due to the acquisition of at least one target mutation in <i>gyrA</i> | A      | Helms <i>et al.</i> , 2002<br>Kadhiravan T <i>et al.</i> , 2005<br>Slinger <i>et al.</i> 2004 |

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

B. Evidence is weak based only on a few cases

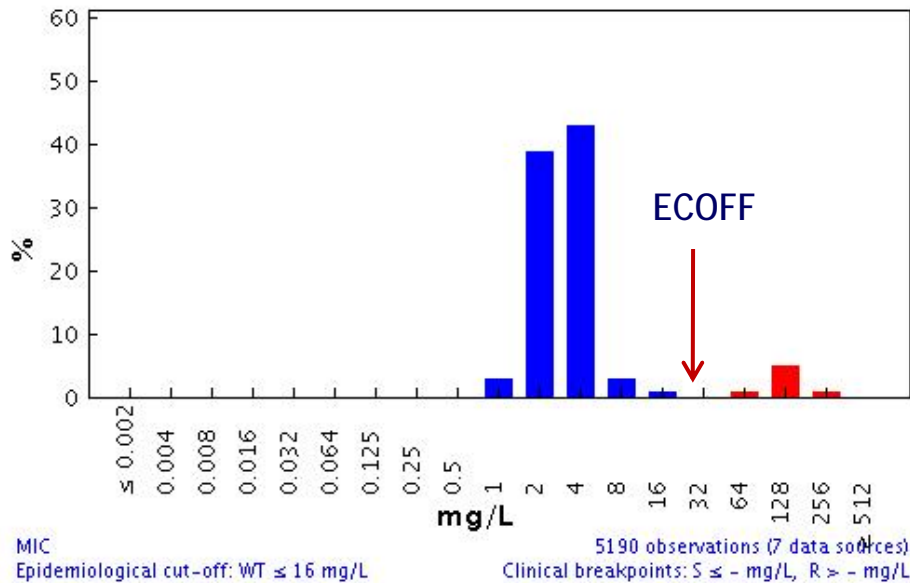
C. There is currently no clinical evidence, but

**Should we apply the *Salmonella* rule (13.7) for all Enterobacteriaceae?**

# Expert rules for quinolones & Enterobacteriaceae

Nalidixic acid / *Escherichia coli*

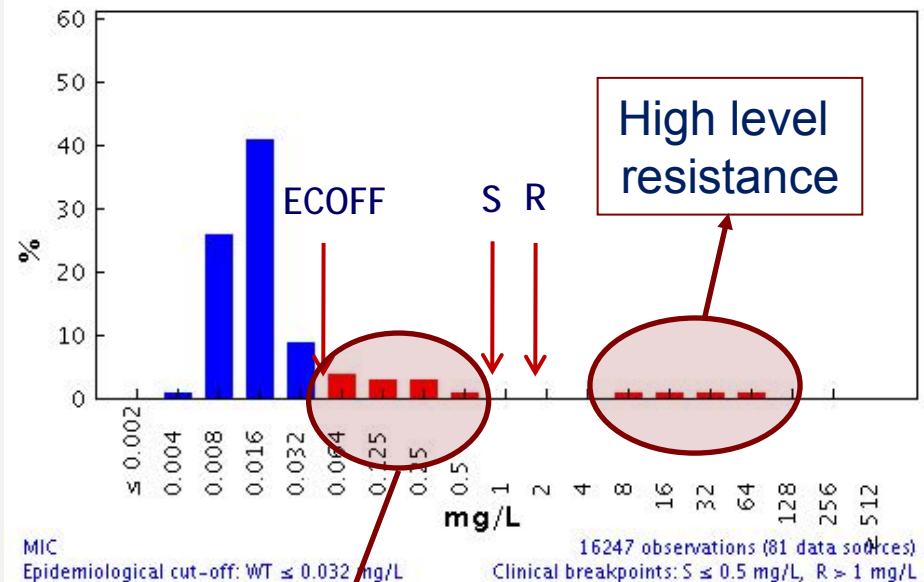
Antimicrobial wild type distributions of microorganisms - reference database  
EUCAST MIC Distribution



ECOFF: epidemiological cut-off values

Ciprofloxacin / *Escherichia coli*

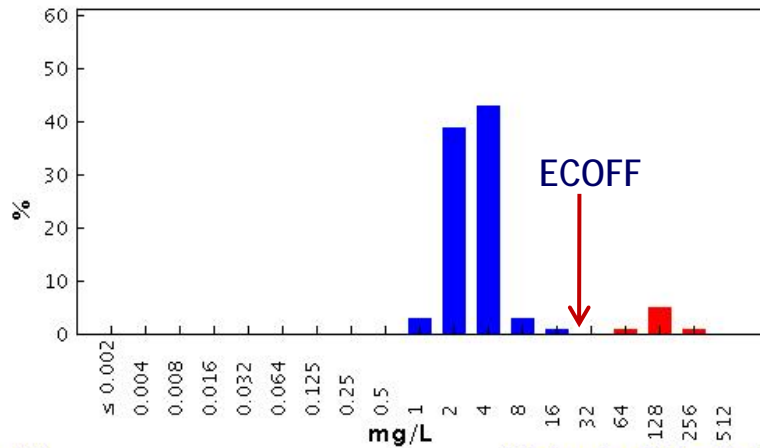
Antimicrobial wild type distributions of microorganisms - reference database  
EUCAST MIC Distribution



Low level resistance?  
Decreased susceptibility?

Nalidixic acid / *Escherichia coli*

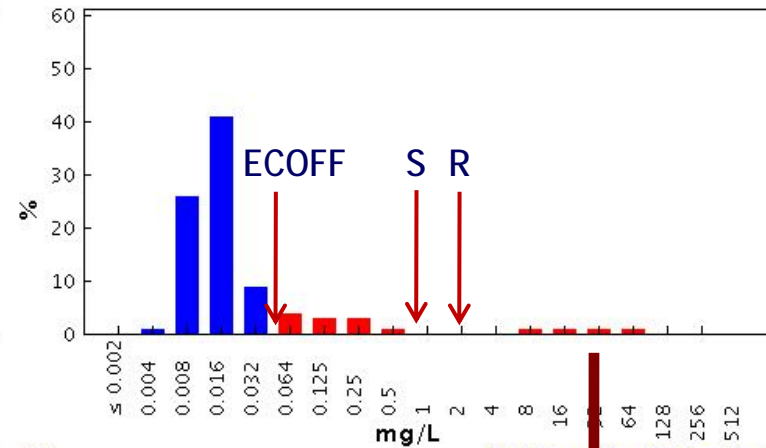
Antimicrobial wild type distributions of microorganisms - reference database  
EUCAST MIC Distribution



MIC Epidemiological cut-off: WT  $\leq$  16 mg/L  
Clinical breakpoints: S  $\leq$  - mg/L, R  $>$  - mg/L  
5190 observations (7 data sources)

Ciprofloxacin / *Escherichia coli*

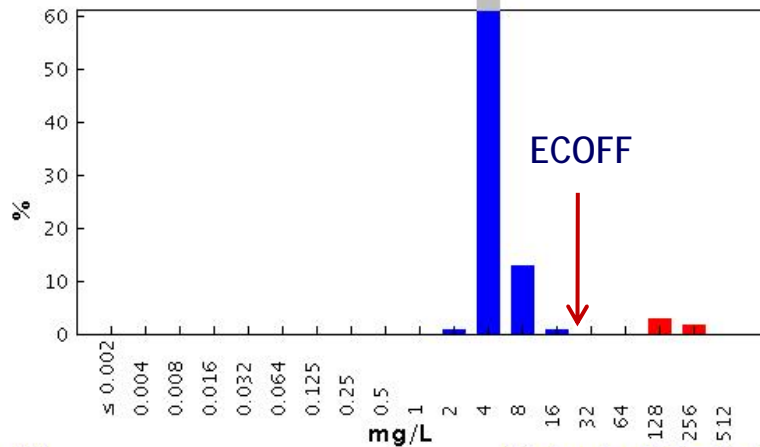
Antimicrobial wild type distributions of microorganisms - reference database  
EUCAST MIC Distribution



MIC Epidemiological cut-off: WT  $\leq$  0.032 mg/L  
Clinical breakpoints: S  $\leq$  0.5 mg/L, R  $>$  1 mg/L  
16247 observations (81 data sources)

Nalidixic acid / *Salmonella spp*

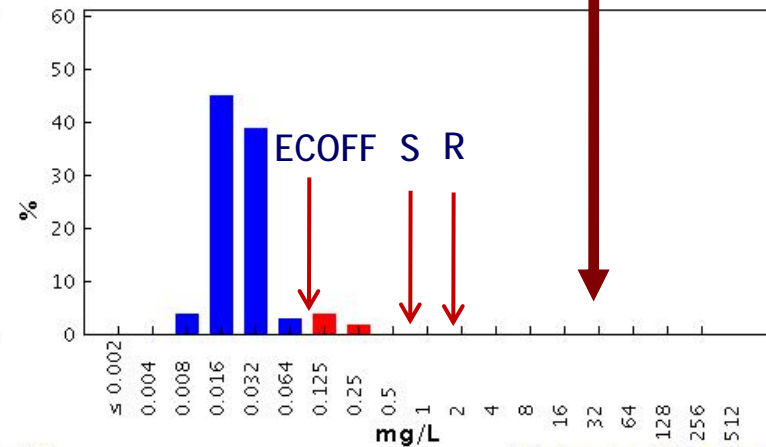
Antimicrobial wild type distributions of microorganisms - reference database  
EUCAST MIC Distribution



MIC Epidemiological cut-off: WT  $\leq$  16 mg/L  
Clinical breakpoints: S  $\leq$  - mg/L, R  $>$  - mg/L  
7143 observations (9 data sources)

Ciprofloxacin / *Salmonella spp*

Antimicrobial wild type distributions of microorganisms - reference database  
EUCAST MIC Distribution



MIC Epidemiological cut-off: WT  $\leq$  0.064 mg/L  
Clinical breakpoints: S  $\leq$  0.5 mg/L, R  $>$  1 mg/L  
1733 observations (3 data sources)

ECOFF: epidemiological cut-off values



# Expert rule for quinolones & Enterobacteriaceae

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- Development of high level resistance to ciprofloxacin occurs at a higher rate in Enterobacteriaceae that are resistant to nalidixic acid but susceptible to ciprofloxacin than in those isolates that are susceptible to both nalidixic acid and ciprofloxacin

| Phenotype      |               | Resistance mechanisms | Resistance development |
|----------------|---------------|-----------------------|------------------------|
| Nalidixic acid | Ciprofloxacin |                       |                        |
| S              | S             | -                     | +                      |
| S(↓)           | S(↓)          | Qnr-like              | ++                     |
| R              | S             | <i>gyrA</i> mutations | +++                    |

- Clinical failure in patients with isolates that are resistant to nalidixic acid has only been adequately documented with *Salmonella* spp. isolates, most of them with low level resistance levels!

# *Salmonella* spp. and quinolone resistance

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- Single point mutations in *gyrA* gene confer resistance (MIC>ECOFF) to nalidixic ac. and low-level resistance to ciprofloxacin
  - isolates can appear as susceptible (MICs = 0.12-1 mg/L)
  - nalidixic ac.<sup>R</sup> is a good marker of low-level ciprofloxacin resistance
- High-level resistance to ciprofloxacin is still scarce comparing with *E. coli* and requires  $\geq 2$  point mutations in *gyrA* gene with ...
  - variably additional mutations in *gyrB*, *parC* or
  - overexpression of *soxR* / *marA* with altered levels of AcrB / OmpF
- Worldwide increment of ciprofloxacin resistance (low- and high-level)

Weinberger . Curr Opin Infect Dis 2005;18: 513-21

Parri. Curr Opin Infec Dis 2003; 16: 467-72

Aarestrup et al. Antimicrob Agents Chemother 2003; 47:827-9

## Treatment failures in patients infected with *Salmonella enterica* serovar Typhi and non-Typhi isolates with decreased susceptibility to fluoroquinolones

| Country        | Serovar        | No. of patients | MIC (mg/L)  |
|----------------|----------------|-----------------|---|
| Denmark        | Enteritidis    | 1               | Original strain, 0.032; after treatment, 1                |
|                | Typhim. DT104  | 27              | 0.064-0.124   |
|                | Typhimurium    | 83              | 0.06-0.38   |
|                | Typhi          | 1               | 0.19  |
| France         | Typhi          | 1               | 0.12  |
| India          | Typhi          | 32              | 0.06-0.5  |
| Spain          | Enteritidis    | 2               | Original strains, 0.06; after treatment, 0.5 and 1        |
| United Kingdom | Typhi          | 1               | 0.5   |
|                | Typhimurium    | 1               | Original strain, 0.03; after treatment, 2.0               |
|                | Typhimurium    | 2               | Original strains, 0.015; 0.03; after treatment, 2; 0.06-1 |
|                | Bovismorbifica | 1               | Original isolate, 0.06; after treatment, 2, 16, and 4     |
|                | Virchow        | 1               | Original strain, 0.016; after initial, treatment, 0.75    |
| Vietnam        | Typhi          | 150             | 0.125-1 (ofloxacin)                                       |

## Clinical Response and Outcome of Infection with *Salmonella enterica* Serotype Typhi with Decreased Susceptibility to Fluoroquinolones: a United States FoodNet Multicenter Retrospective Cohort Study<sup>▽</sup>

John A. Crump,<sup>1,4\*†</sup> Katrina Kretsinger,<sup>1,4†</sup> Kathryn Gay,<sup>2</sup> R. Michael Hoekstra,<sup>3</sup> Duc J. Vugia,<sup>5</sup> Sharon Hurd,<sup>6</sup> Susan D. Segler,<sup>7</sup> Melanie Megginson,<sup>8</sup> L. Jeffrey Luedeman,<sup>9</sup> Beletshachew Shiferaw,<sup>10</sup> Samir S. Hanna,<sup>11</sup> Kevin W. Joyce,<sup>2</sup> Eric D. Mintz,<sup>1</sup> Frederick J. Angulo,<sup>1</sup> and the Emerging Infections Program FoodNet and NARMS Working Groups

|                          | Suceptible<br>to ciprofloxacin<br>(<0.12 mg/L) | Decreased susceptibility<br>to ciprofloxacin<br>(0.12-1 mg/L) |
|--------------------------|--|---|
| Antimicrobial-related    |  |   |
| fever clearance time (h) | 72 (19-264)                                    | 92 (21-373)   |
| Ciprofloxacin-related    |  |   |
| fever clearance time (h) | 64 (34-204)                                    | 90 (9-373)  |
| Treatment failure        | 4% (2/46)                                      | 17% (4/24)  |

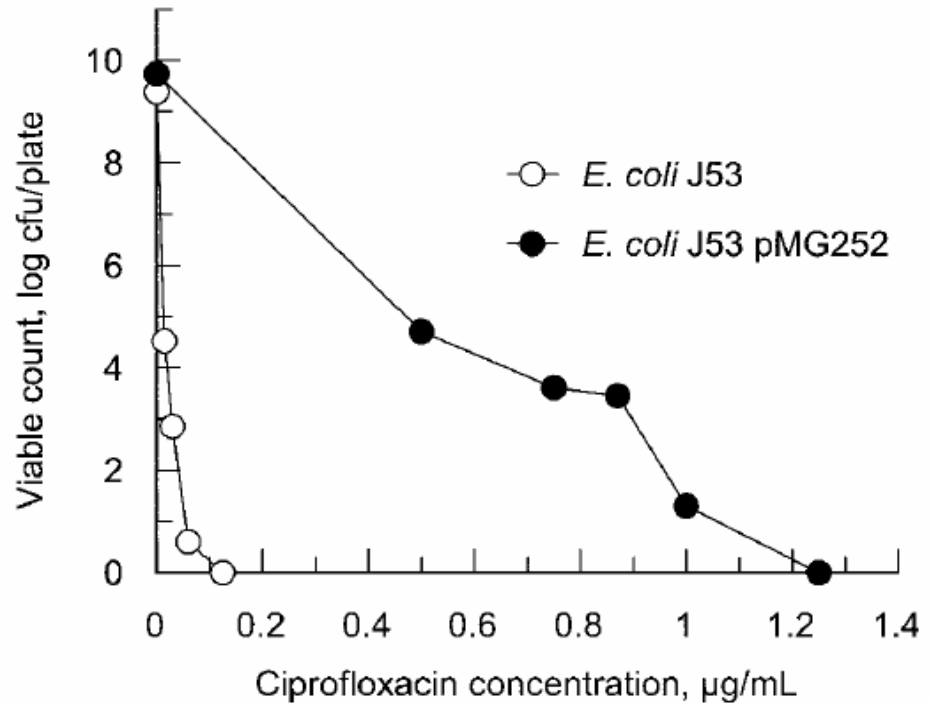
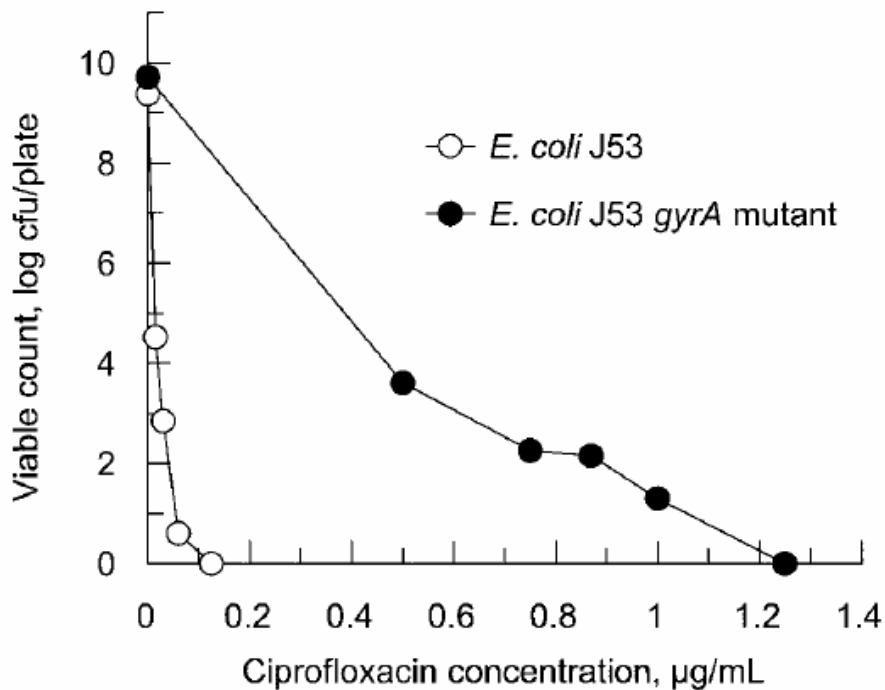
# *Escherichia coli*: fluoroquinolone resistance

| Mutation in: |             |        | MIC ( $\mu\text{g/ml}$ ) |      |      |      |  |
|--------------|-------------|--------|--------------------------|------|------|------|--|
| <i>gyrA</i>  | <i>parC</i> | Efflux | NAL                      | CIP  | LEV  | MOX  |  |
| -            | -           | -      | 2                        | 0.01 | 0.06 | 0.06 |  |
| +            | -           | -      | 32-256                   | 0.5  | 0.5  | 1    |  |
| -            | +           | -      | 64                       | 0.01 | 0.03 | 0.2  |  |
| +            | +           | -      | >1024                    | 1    | 2    | 2    |  |
| +            | -           | +      | 32->1024                 | 2    | 4    | 4    |  |
| +            | +           | +      | >1024                    | 64   | 32   | 32   |  |
| +            | +           | +      | >1024                    | 256  | 64   | 128  |  |

CIP: ciprofloxacin; LEV: levofloxacin; MOX: moxifloxacin

# *Escherichia coli*: low level fluoroquinolone resistance

*In vitro* effect of the *gyrA* mutation or *qnr* presence on the recovery of ciprofloxacin-resistant mutant



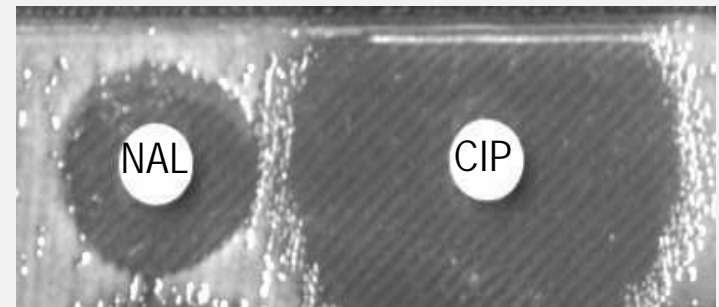
# Low level fluoroquinolone resistance

## *Escherichia coli* phenotypes

| Resistance mechanisms | MIC (mg/L) |            |           |           |
|-----------------------|------------|------------|-----------|-----------|
|                       | NAL        | CIP        | LEV       | MOX       |
| Wild type             | 2-4        | 0.008-0.02 | 0.08-1    | 0.03      |
| QnrA                  | 8-32       | 0.12-2     | 0.25-0.5  | 0.5-1     |
| QnrB                  | 16         | 0.25-1     | 0.5       | 1-2       |
| QnrS                  | 8-32       | 0.12-0.5   | --        | 0.25      |
| AAC(6')-Ib-cr         | --         | 0.08       | 0.08      | --        |
| QepA                  | 1-2        | 0.25       | 0.03-0.06 | 0.06-0.09 |

NAL: nalidixic acid; CIP: ciprofloxacin; LEV: levofloxacin; MOX: moxifloxacin

- MICs not always higher than ECOFF (16 mg/L) for nalidixic acid
- MICs for ciprofloxacin higher than ECOFF (0.032 mg/L) but lower than S breakpoint (0.5 mg/L)



[www.seimc.org](http://www.seimc.org)

Robisek et al. Nat Med 2006; 12:83-88

Robicsek et al. Lancet Infect Dis 2006; 6:629-40

Yamane et al. Antimicrob Agents Chemother 2007; 51:3354-60

## Mutant Prevention Concentrations of Fluoroquinolones for *Enterobacteriaceae* Expressing the Plasmid-Carried Quinolone Resistance Determinant *qnrA1*<sup>∇</sup>

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- MPC are always higher in *qnrA1*-(+) than *qnrA1*-(-) isolates
- Quinolone-R mutants in *qnrA1*-(+) isolates emerge in a short period of time and in a low quantity when compared with *qnrA1*-(-)

|                      | <i>qnrA1</i> -(-) | <i>qnrA1</i> -(+) |
|----------------------|-------------------|-------------------|
| <i>E. coli</i>       | 0.015-0.125*      | 2-4               |
| <i>K. pneumoniae</i> | 4-8               | 4-128             |


\*mg/L

## In Vivo Selection of Fluoroquinolone-Resistant *Escherichia coli* Isolates Expressing Plasmid-Mediated Quinolone Resistance and Expanded-Spectrum $\beta$ -Lactamase

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- Selection of a CIP<sup>R</sup> *E. coli* isolate during treatment with norfloxacin of a UTI due to an ESBL (VEB-1) and QnrA1CIP<sup>S</sup> *E. coli* isolate

|               | <i>E. coli</i><br>before treatment |  | <i>E. coli</i><br>after treatment |
|---------------|------------------------------------|--|-----------------------------------|
| Nalidixic ac. | 16*                                |  | >256                              |
| Norfloxacin   | 2                                  |  | >256                              |
| Ciprofloxacin | 0.5                                |  | >32                               |
| GyrA mutation | Wild type                          |  | Ser83Leu / Asp87Asn               |
| ParC mutation | Wild type                          |  | Ser80II                           |

\*mg/L

**EUCAST interpretive rules in  
antimicrobial susceptibility testing for  
fluoroquinolones and aminoglycosides  
and Enterobacteriaceae**

**EUCAST EDUCATIONAL WORKSHOP**  
*18th ECCMID Barcelona, Spain, 2008*

Rafael Cantón

**Servicio de Microbiología**



**Hospital Ramón y Cajal**



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ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING