

# Resistensbestämning med fenotypiska metoder baseras på MIC

**Gunnar Kahlmeter**

Clinical microbiology  
Kronoberg och Blekinge, Sweden

# Methods for susceptibility testing

- **Phenotypic test methods**

based on antimicrobial activity (**MIC**) and **breakpoints**

- Disk diffusion, automated systems like Phoenix, Vitek2, Microscan, Gradient tests
- Predict susceptibility and resistance
- Quantifiable

- **Genotypic test methods**

based on the detection of a **resistance gene** or its **product (PCR, WGS)**

- *mecA*, *vanA*, *vanB*, ....PBP2, ... betalactamase detection (enzyme detection, Maldi Tof)
- Predict resistance, not sensitivity
- Not quantifiable
- Useful for epidemiological purposes.

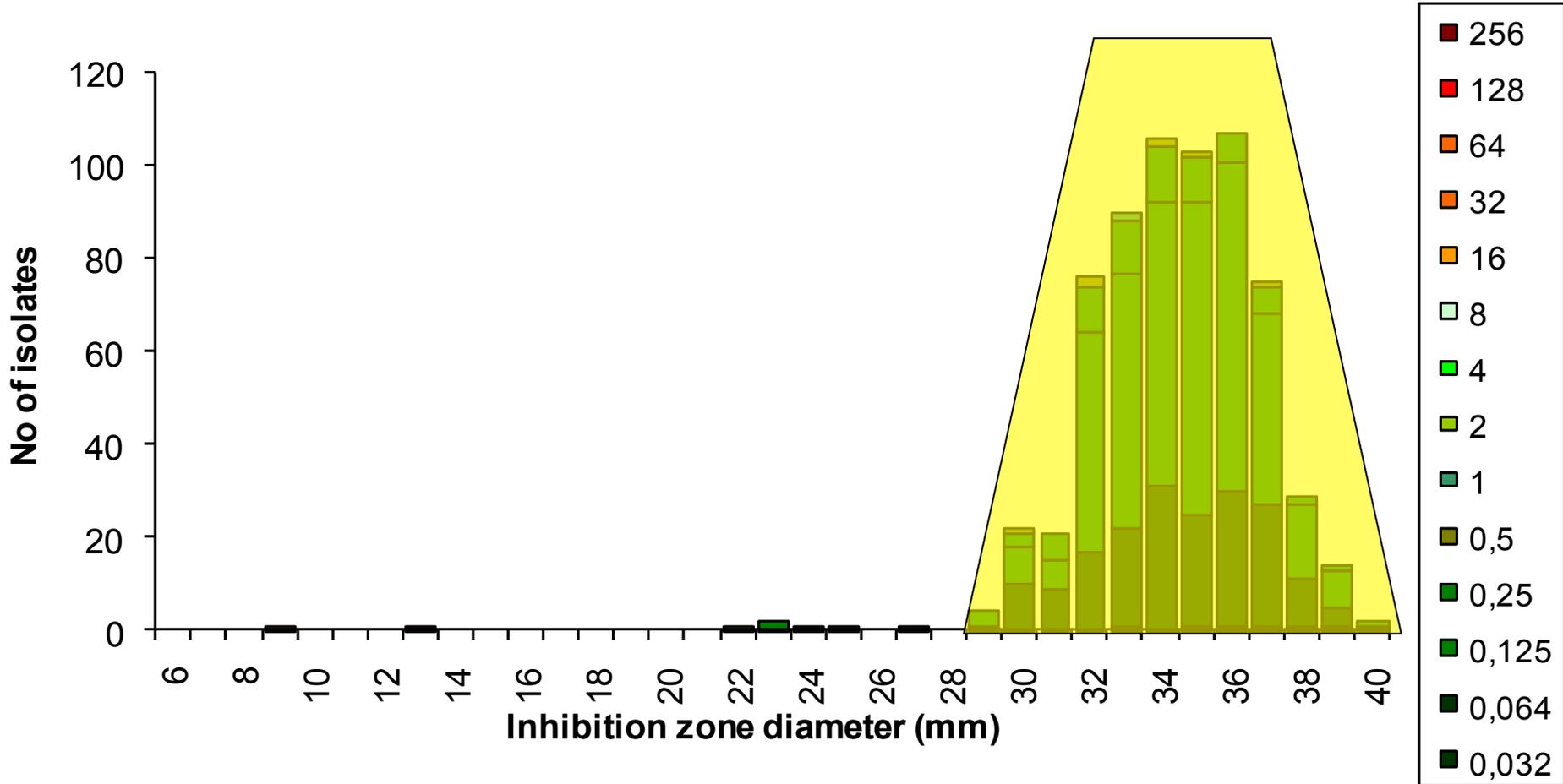
- **By deduction – “expert rules”**

- If MRSA then report all betalactam antibiotics R – or soon not?  
If ESBL-positive, then report betalactam antibiotics R – but not any longer!  
If erythromycin-resistant, then report all macrolide antibiotics as R;
- **Some rules predict susceptibility, others resistance.**
- **Not quantifiable.**
- **Unreliable !**



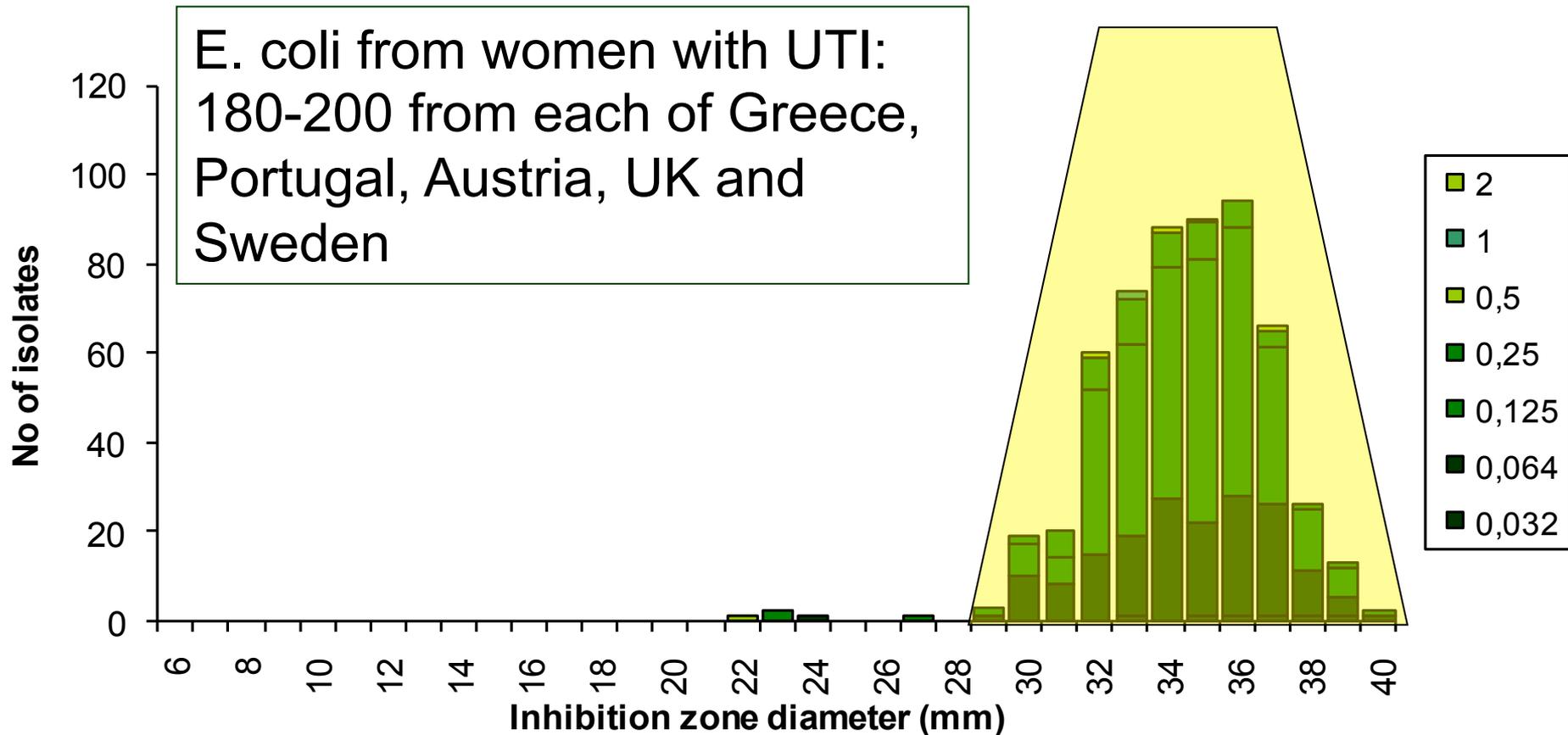


***E. coli* vs. *mecillinam***  
**657 isolates susceptible to ampicillin**



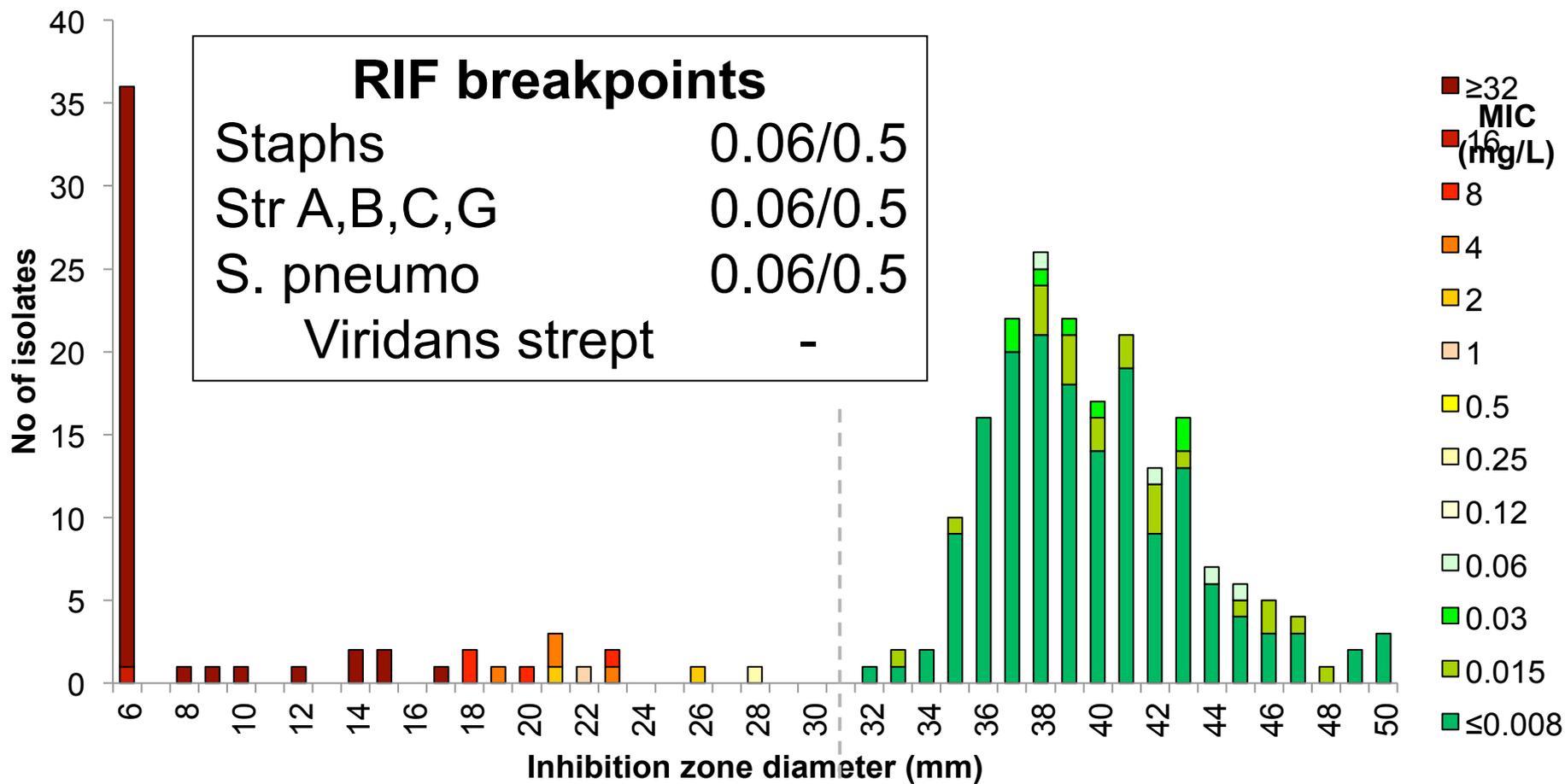
## *E. coli* vs. *mecillinam*

560 isolates susceptible to all agents tested



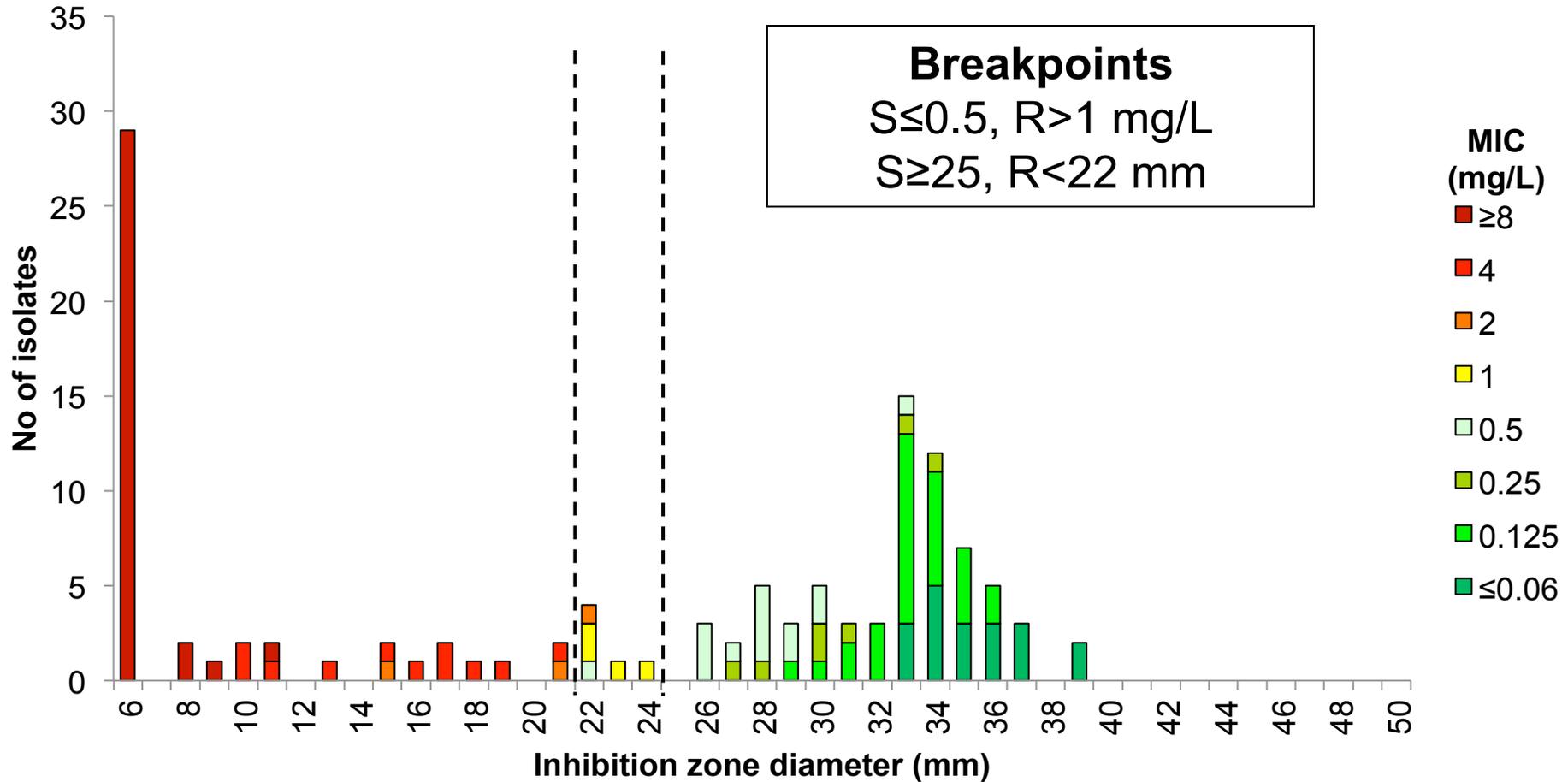
# Rifampicin 5 $\mu$ g vs. MIC

## *Corynebacterium* spp, 253 clinical isolates



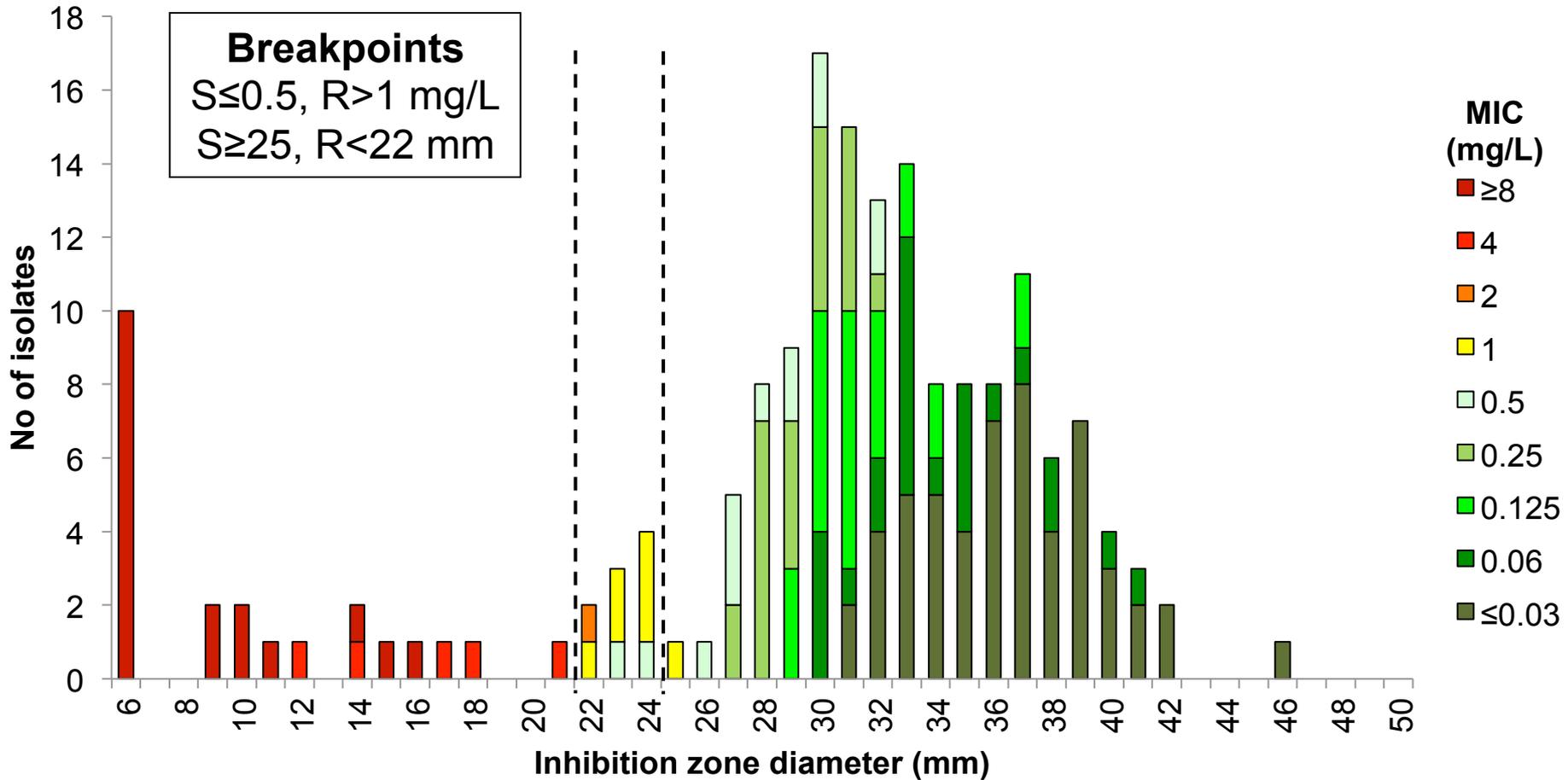
# Ciprofloxacin 5 $\mu$ g vs. MIC

## *Pseudomonas aeruginosa*, 120 clinical isolates



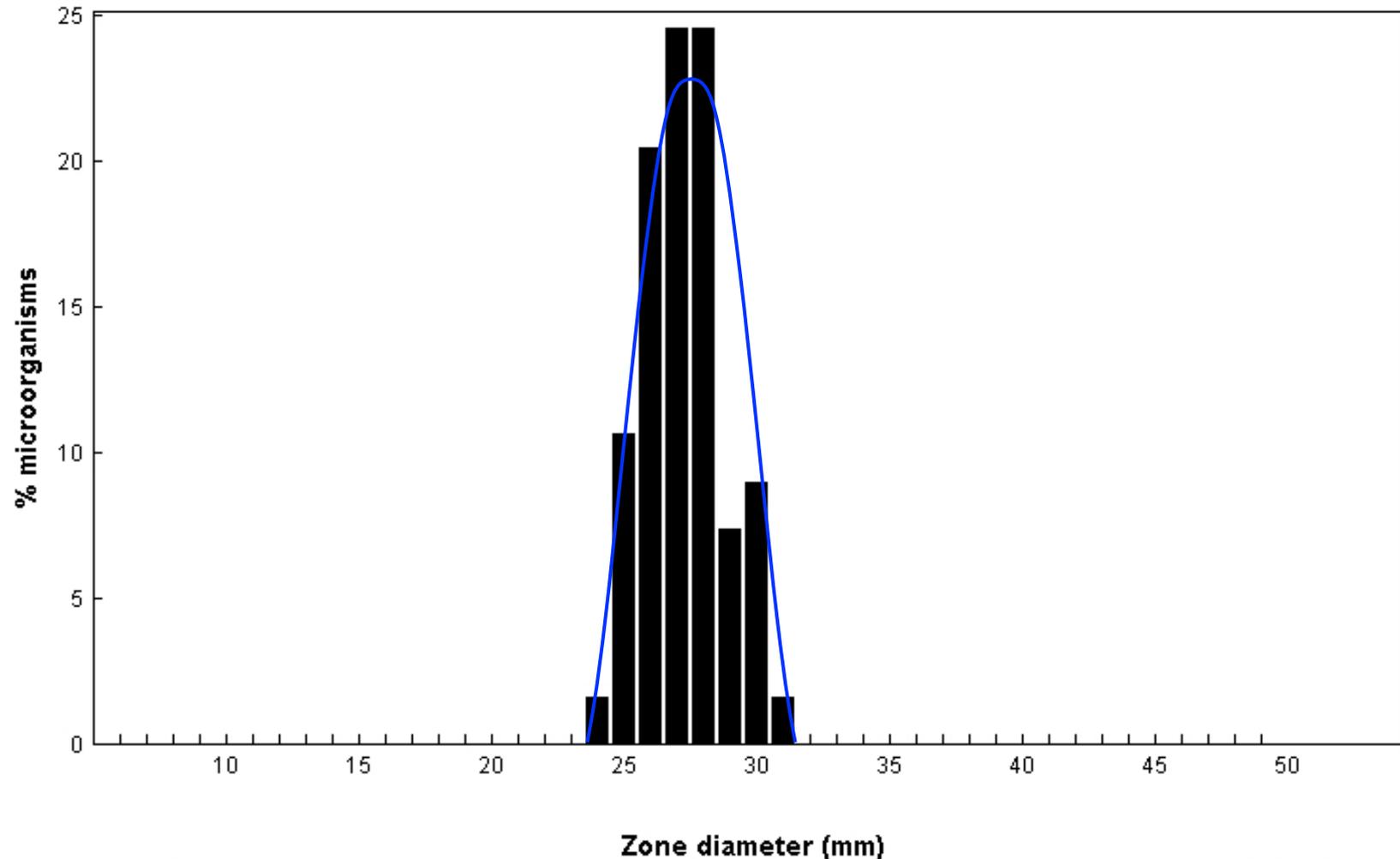
# Ciprofloxacin 5 $\mu$ g vs. MIC

## *Pseudomonas non-aeruginosa*, 173 clinical isolates



**Cefoxitin / Staphylococcus aureus ATCC 29213**  
**EUCAST zone diameter distribution - Reference database**  
**EUCAST disk diffusion method**

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 30

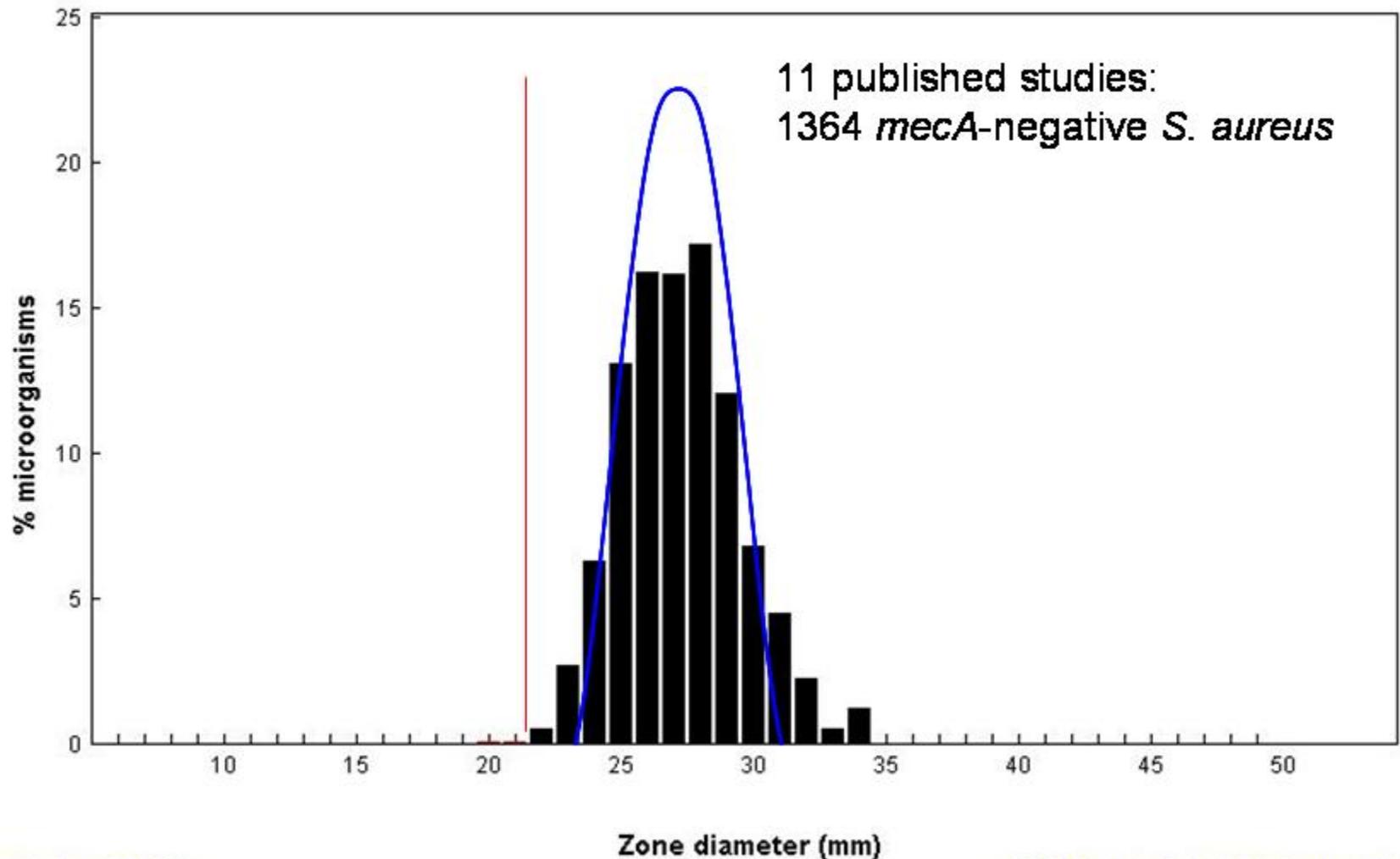
Epidemiological cut-off: WT  $\geq$  22 mm (MIC: -)

122 observations

Clinical breakpoints: S  $\geq$  22 mm, R  $<$  22 mm

**Cefoxitin / Staphylococcus aureus MSSA**  
**EUCAST zone diameter distributions - Reference database**  
**EUCAST disk diffusion method**

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

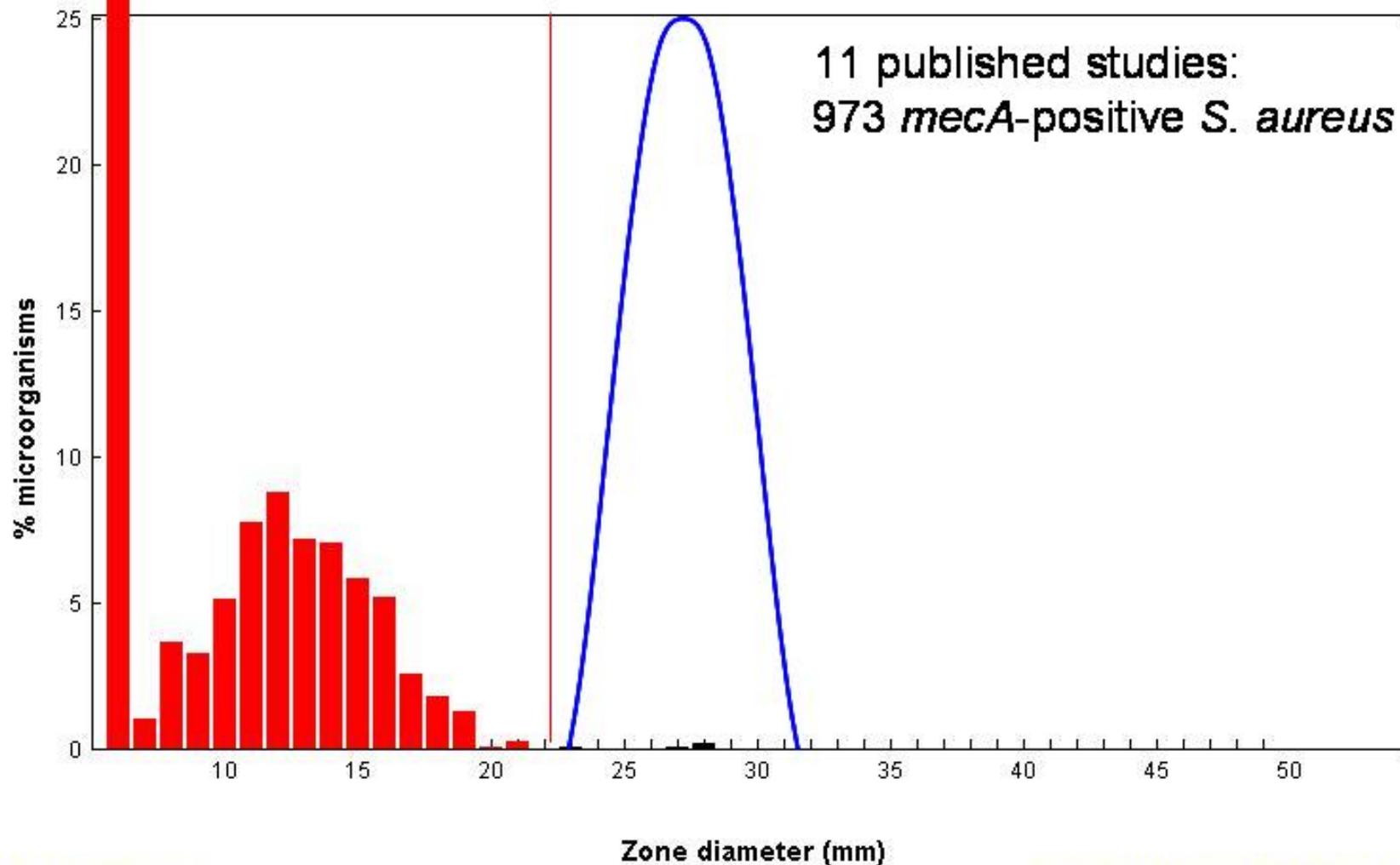


Disk content: 30  
Epidemiological cut-off: WT  $\geq$  22 mm (MIC:  $\leq$  4 mg/L)

1364 observations (11 data sources)  
Clinical breakpoints: S  $\geq$  22 mm, R  $<$  22 mm

**Cefoxitin / Staphylococcus aureus MRSA**  
**EUCAST zone diameter distributions - Reference database**  
**EUCAST disk diffusion method**

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

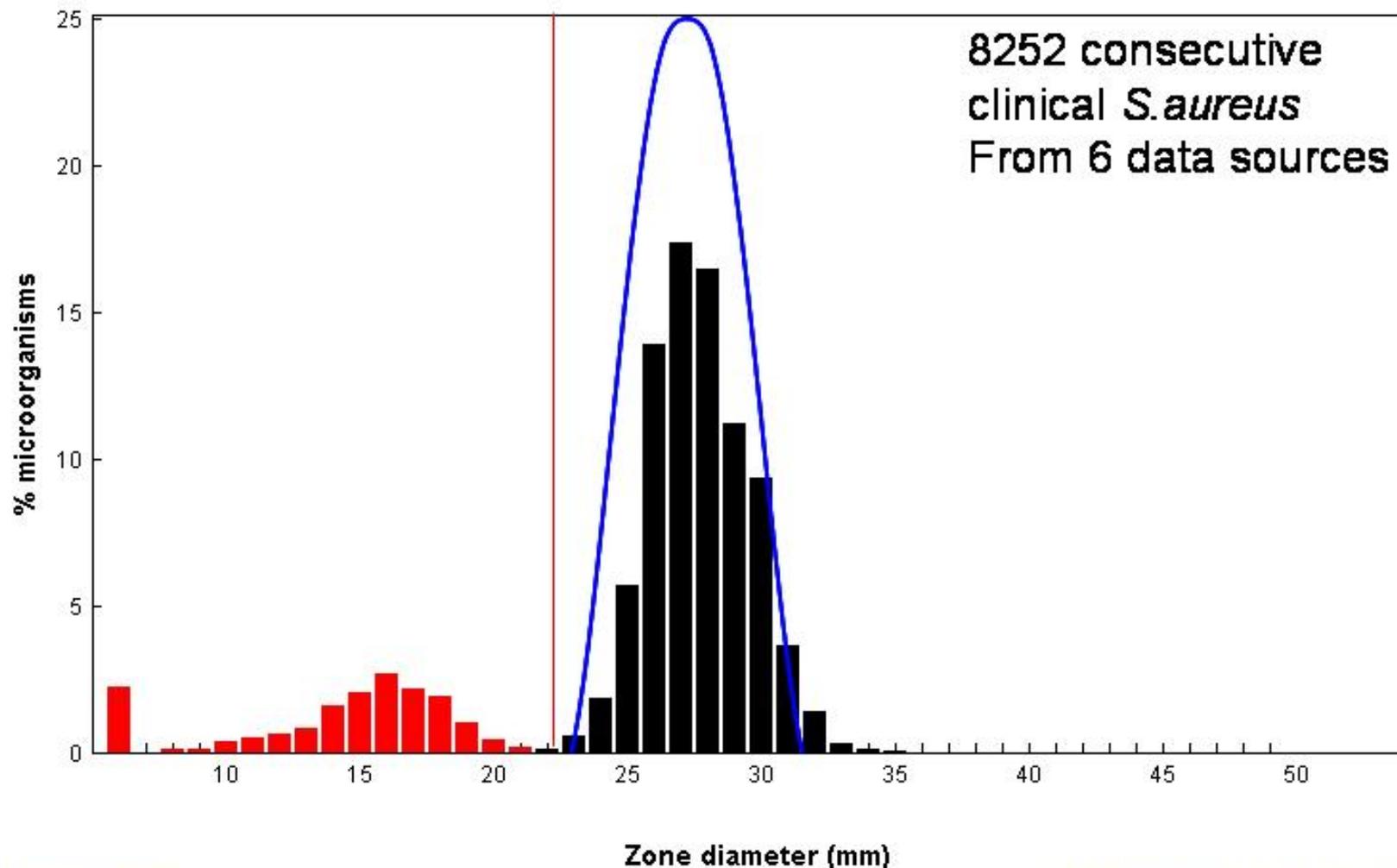


Disk content: 30  
Epidemiological cut-off: WT  $\geq$  22 mm (MIC:  $\leq$  4 mg/L)

973 observations (11 data sources)  
Clinical breakpoints: S  $\geq$  22 mm, R < 22 mm

**Cefoxitin / *Staphylococcus aureus***  
**EUCAST zone diameter distribution - Reference database 2011-01-25**  
**EUCAST disk diffusion method**

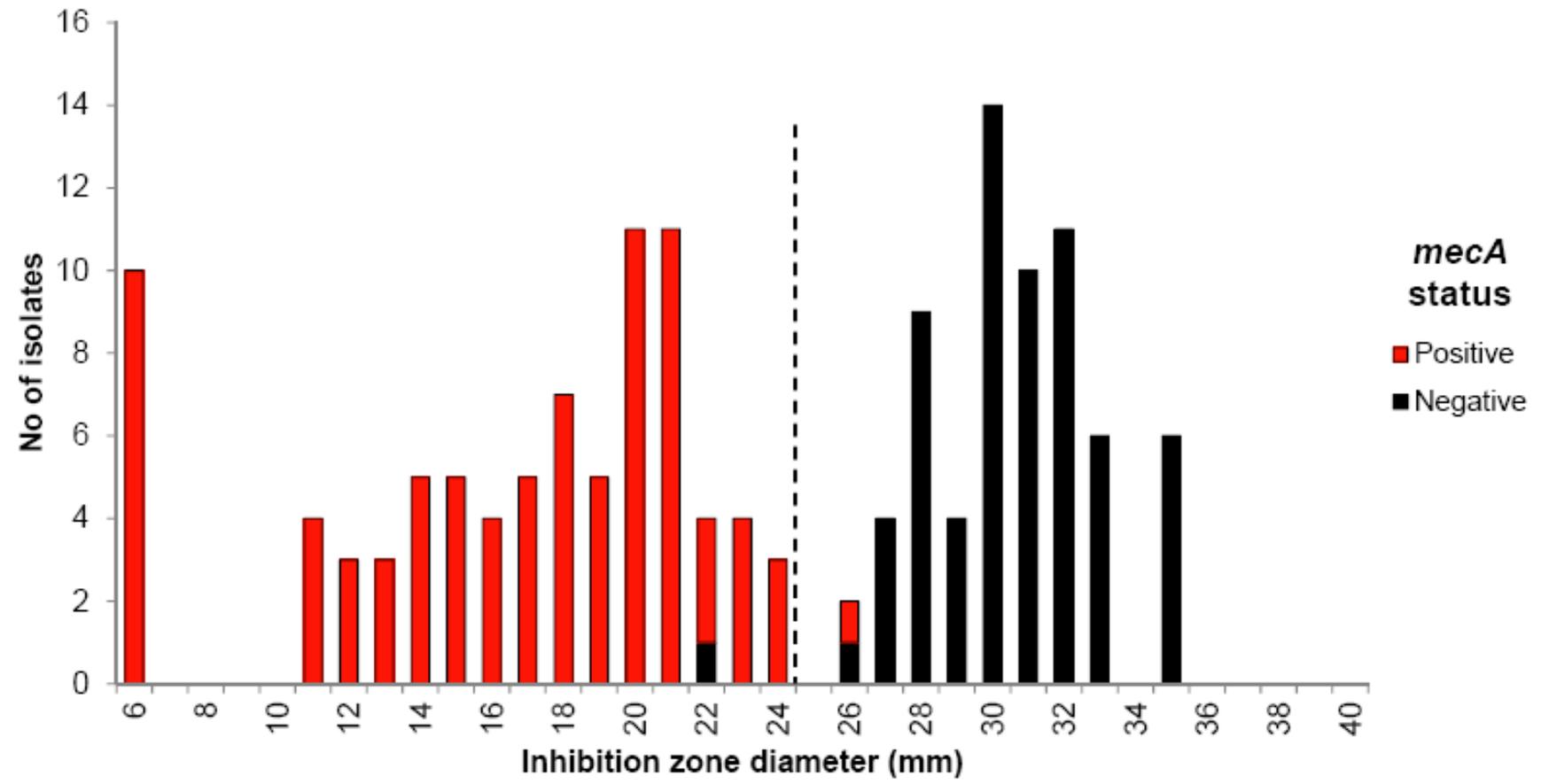
Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 30  
Epidemiological cut-off: WT  $\geq 22$  mm (MIC:  $\leq 4$  mg/L)

8252 observations (6 data sources)  
Clinical breakpoints: S  $\geq 22$  mm, R  $< 22$  mm

# Cefoxitin 30 µg vs. *mecA* status CoNS, 150 clinical isolates



**Breakpoints**  
Zone diameter (screen)     $S \geq 25$ ,  $R < 25$  mm

# MIC

- MIC – the minimum inhibitory concentration  
– mg/L or  $\mu\text{g/mL}$
- The lowest concentration in a series of two-fold concentrations that will inhibit the growth of a microorganism, as measured by the **naked** eye.
- Convention: The concentration series shall contain the concentration **1** mg/L

0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.12,  
0.25, 0.5, **1**, 2, 4, 8, 16, 32, 64, 128, 256, 512

# MIC

**in vitro**

**relative**

# MIC

- Detailed standardisation (ISO-document) of a test system gives accurate and reproducible results.
- Accurate = the agreed result
- Accurate  $\neq$  correct
  - unless “correct” is defined as the “agreed result”)

# ISO 20776-1 (2006)

- Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices –  
**Part 1:** Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.
- Broth microdilution technique.
- Rapidly growing aerobic bacteria involved in infectious diseases.
- 2.5-5% lysed horse blood for *Streptococcus* spp.
- No recommendations for *Haemophilus*, anaerobes or other fastidious organisms.

# Accurate = the agreed result

## *Pseudomonas aeruginosa* ATCC 27853

(NCTC 12903, CIP 76.110, DSM 1117, CCUG 17619, CECT 108)

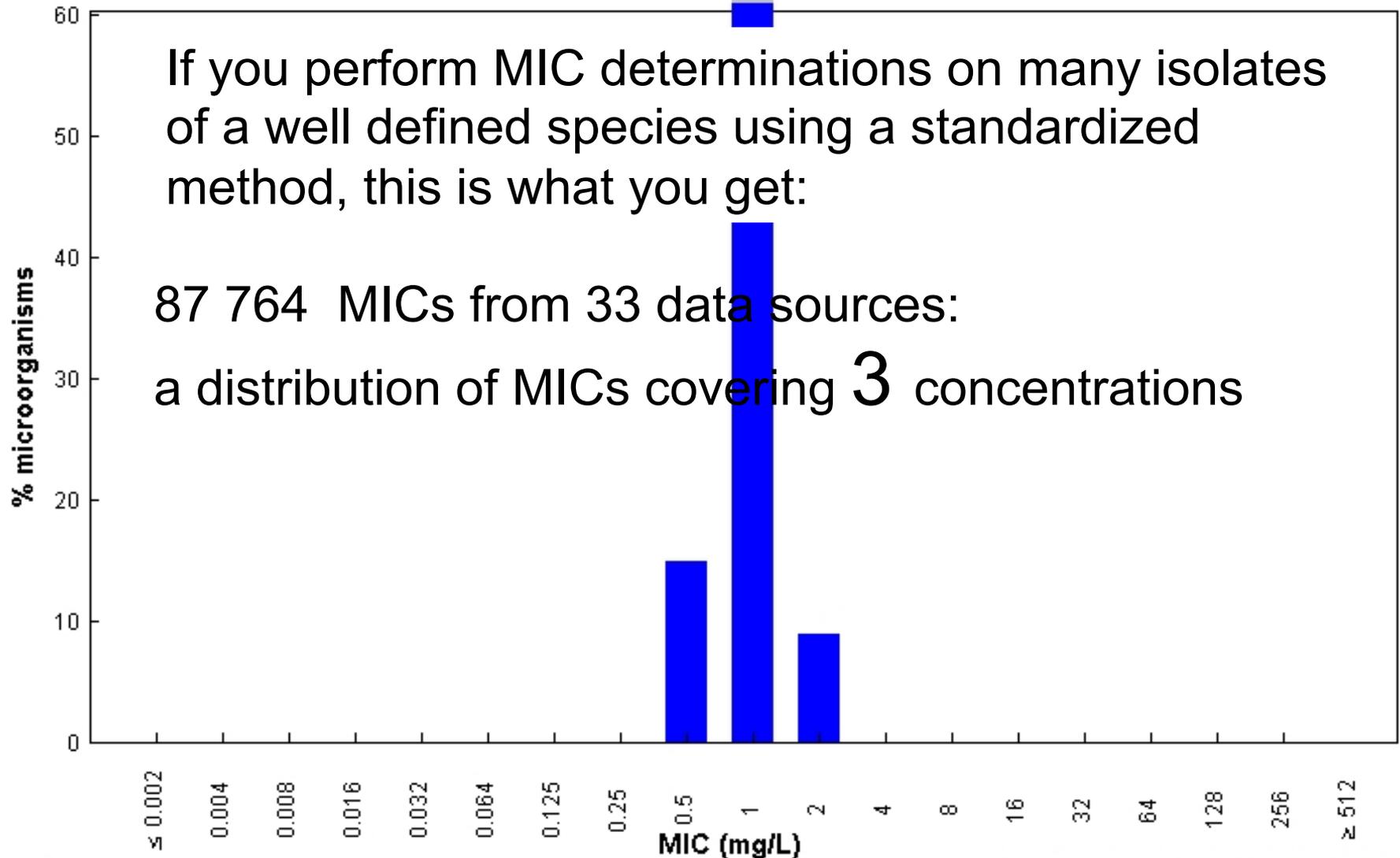
Had we manipulated the conditions (medium, pH, incubation time, inoculum, cation... etc) target values and ranges would be different.

Levofloxacin	1-2	0.5-4	5	23	19-26
Meropenem	0.5	0.25-1	10	30	27-33
Netilmicin	2	0.5-8	10	<b>18</b>	<b>15-21</b>
Piperacillin	2-4	1-8	-	-	-
Piperacillin-tazobactam	2-4/4	1/4-8/4	30-6	<b>26</b>	<b>23-29</b>
Ticarcillin	16	8-32	-	-	-
Ticarcillin-clavulanic acid	16/2	8/2-32/2	75-10	24	20-28
Tobramycin	0.5	0.25-1	10	23	20-26



**Vancomycin / Staphylococcus aureus**  
**EUCAST MIC Distribution - Reference Database 2011-04-21**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC  
Epidemiological cut-off: WT ≤ 2 mg/L

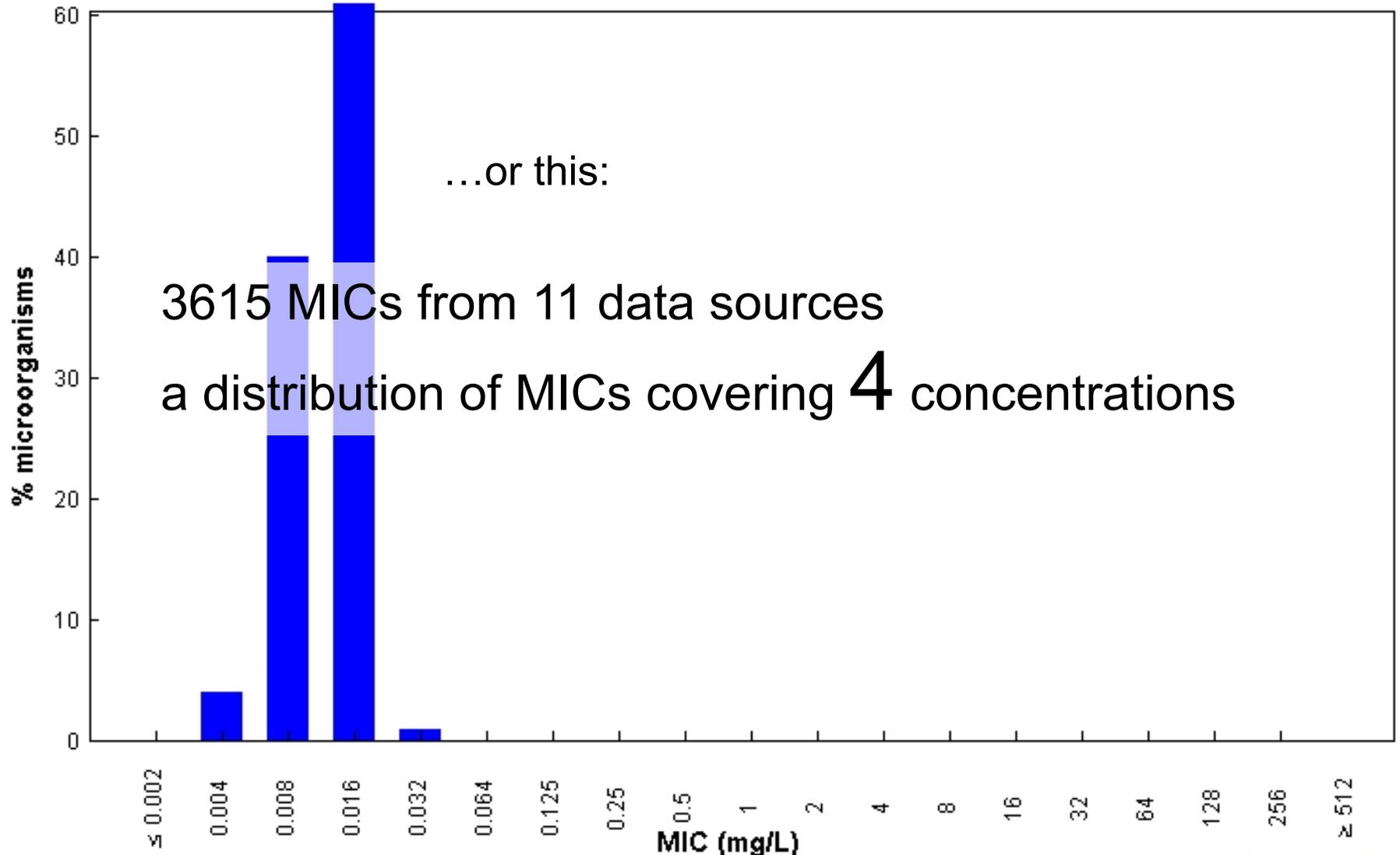
87764 observations (33 data sources)  
Clinical breakpoints: S ≤ 2 mg/L, R > 2 mg/L

**Benzylpenicillin / *Streptococcus pyogenes***  
**EUCAST MIC Distribution - Reference Database**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

...or this:

3615 MICs from 11 data sources  
a distribution of MICs covering 4 concentrations

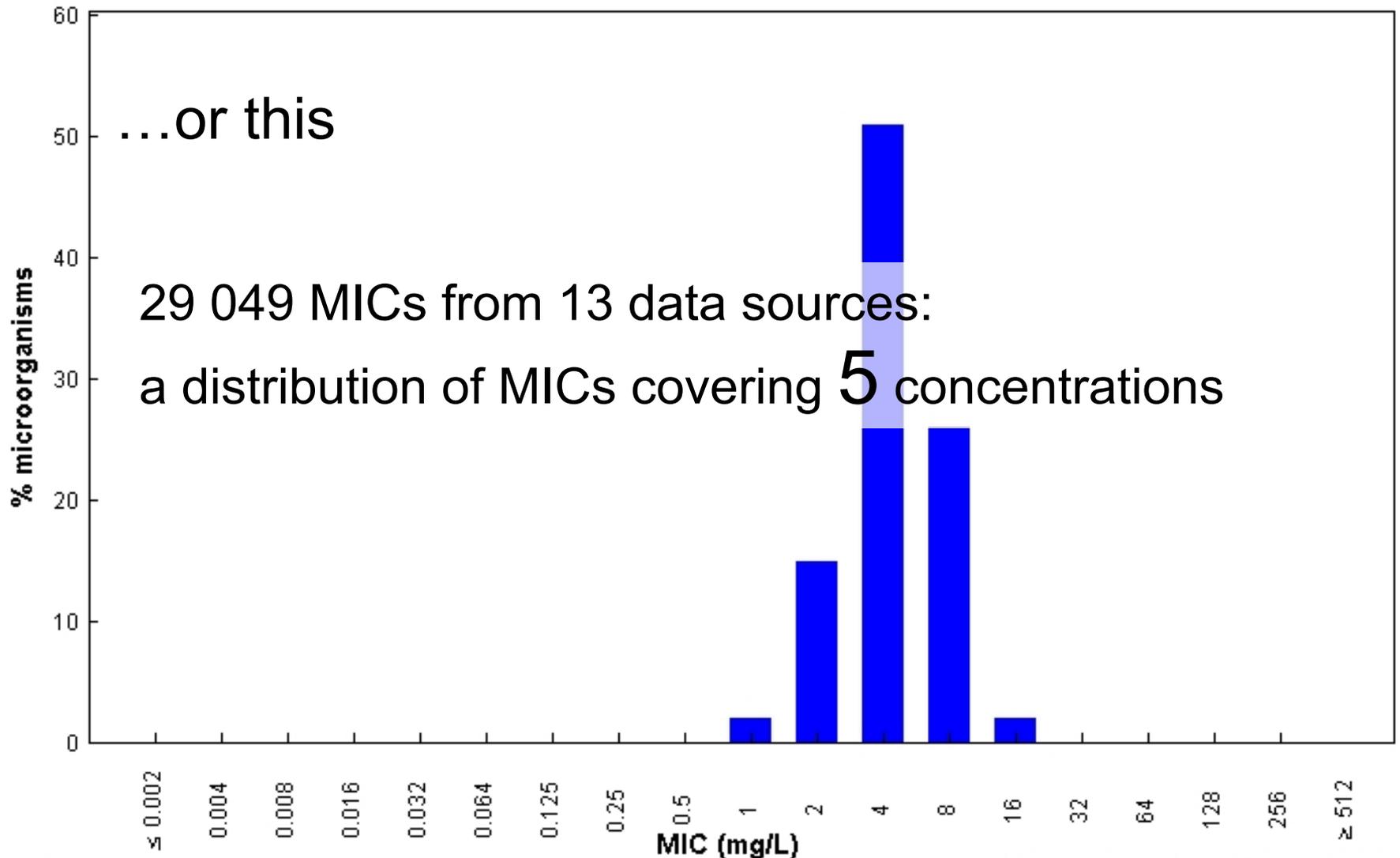


MIC  
Epidemiological cut-off: WT ≤ 0.064 mg/L

3615 observations (11 data sources)  
Clinical breakpoints: S ≤ 0.25 mg/L, R > 0.25 mg/L

Erythromycin / Haemophilus influenzae  
EUCAST MIC Distribution - Reference Database 2011-04-25

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

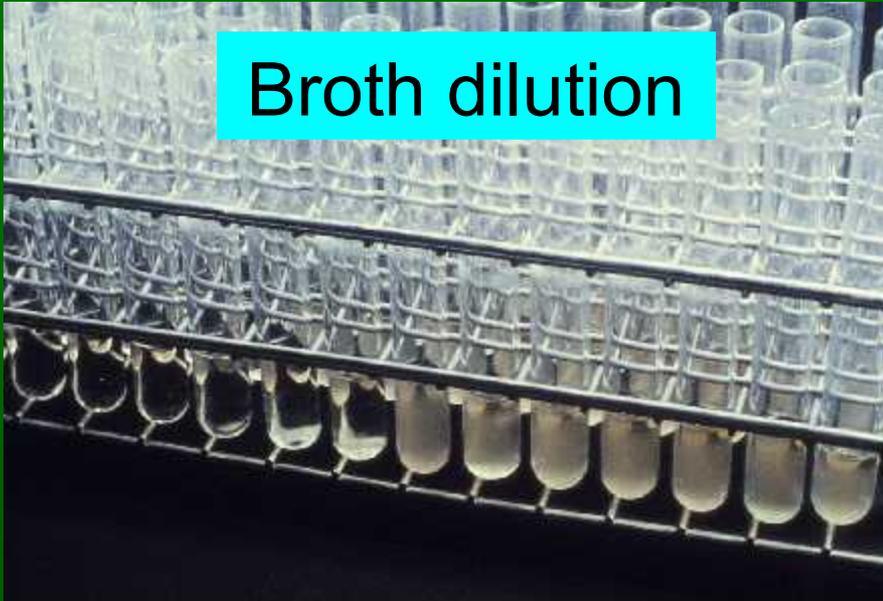


MIC  
Epidemiological cut-off: WT ≤ 16 mg/L

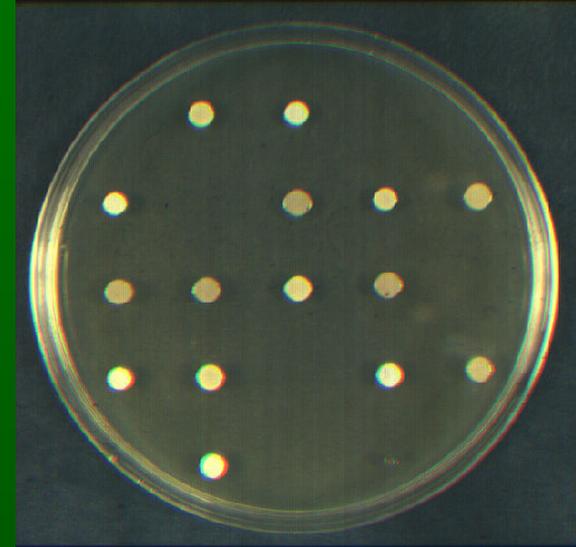
29049 observations (13 data sources)  
Clinical breakpoints: S ≤ 0.5 mg/L, R > 16 mg/L

# Methods for MIC determination

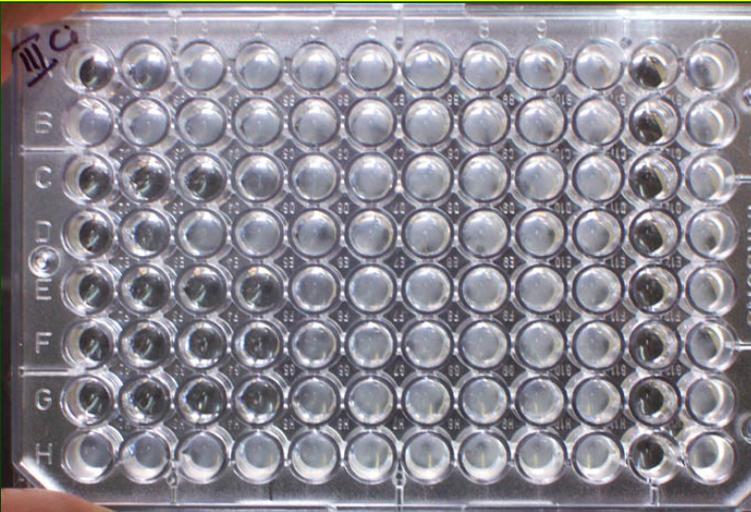
Broth dilution



Agar dilution



Broth microdilution (BMD)



Gradient MIC test

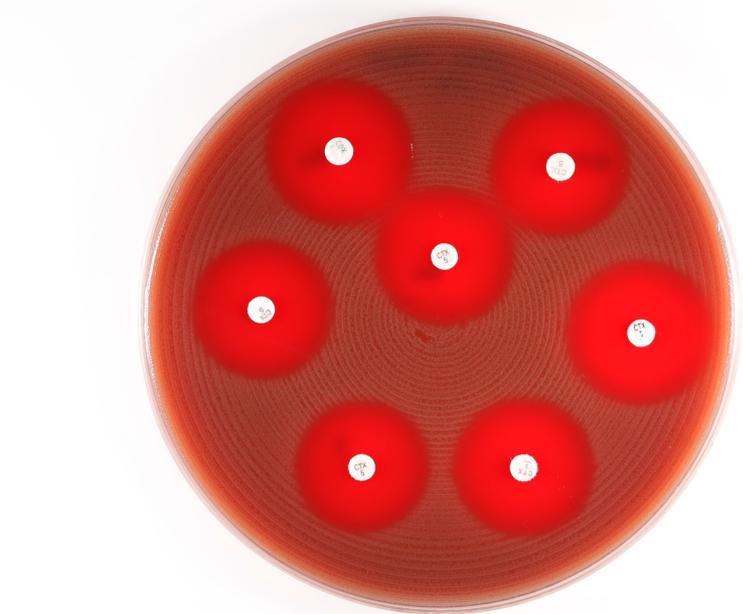
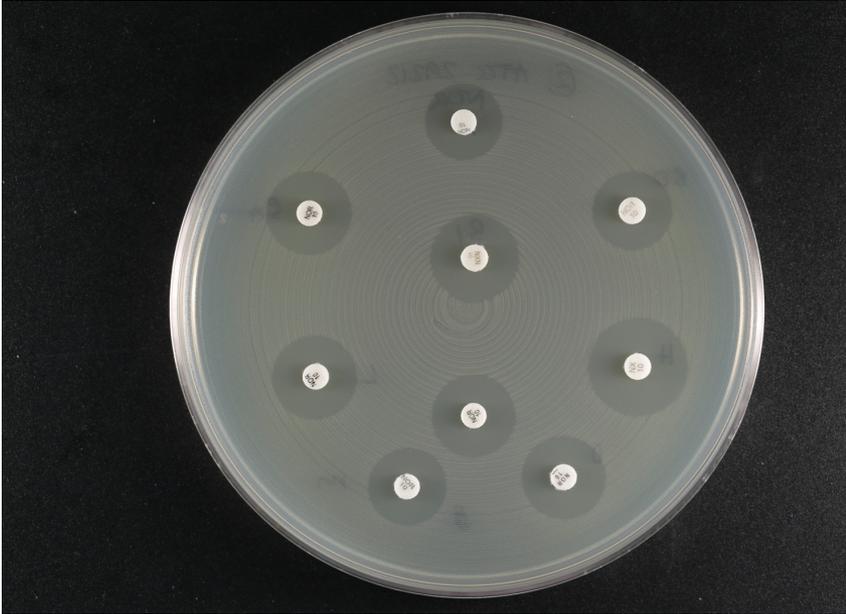
Several manufacturers:

bioMérieux

Oxoid

Liofilchem









# Methods for MIC determination

- Broth dilution
- Broth microdilution (BMD)
  - Several systems available commercially
- Agar dilution
- Gradient tests
  - Several available commercially (Etest, M.I.C.E., MIC Test Strip)

16 8 4 2 1 .5 .25 .12 .06 .03 C

Skov, Matuschek & Kahlmeter

Pefloxacin as screen for low  
level ciprofloxacin resistance in  
Salmonella

# Features of MIC dilution methods

	Broth MIC	Agar MIC	Gradient test	Disk diffusion
Contamination detected	No	Yes	Yes	Yes

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Inoculum effect - suphonamides - beta-lactamase	+++	+	+	(+++)

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	Broth MIC	Agar MIC	Gradient test	Disk diffusion
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Useful for slow- growing (>24 - 48 h) organisms	+++	+++	++	-

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	Broth MIC	Agar MIC	Gradient test	Disk diffusion
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Useful for slow- growing (>24 h) organisms	+++	+++	++	-
Direct AST possible (clin material)	No	?	Yes	Yes

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Inoculum effect - suphonamides - beta-lactamase	+++	+	+	(+++)
Useful for slow- growing (>24 h) organisms	+++	+++	++	-
Direct AST possible (clin material)	No	?	Yes	Yes
Automation	Yes	Partial	No	(Partial)

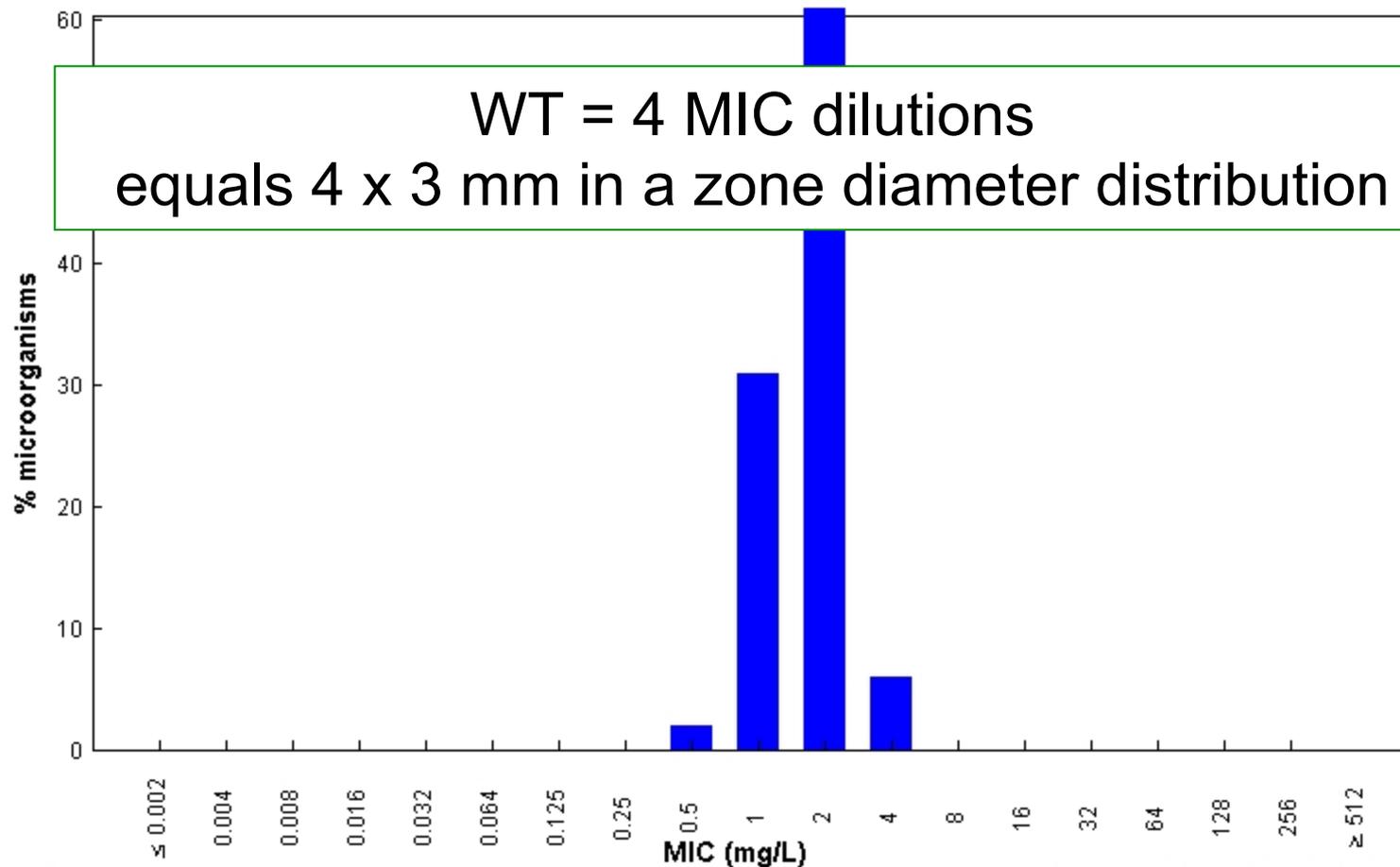
# Automated systems

- **Broth microdilution**
  - Limited dilution series (at worst two concentrations)
    - “Black box” results - often not true MIC-values
    - No easy change in breakpoints
    - Many results reported as  $\leq X$  mg/L or  $> Y$  mg/L
    - A challenge to QC
- **Gradient tests** – semiautomated ??
- **Disk diffusion** – semiautomated ??



Linezolid / *Staphylococcus aureus*  
EUCAST MIC Distribution - Reference Database 2011-05-06

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC

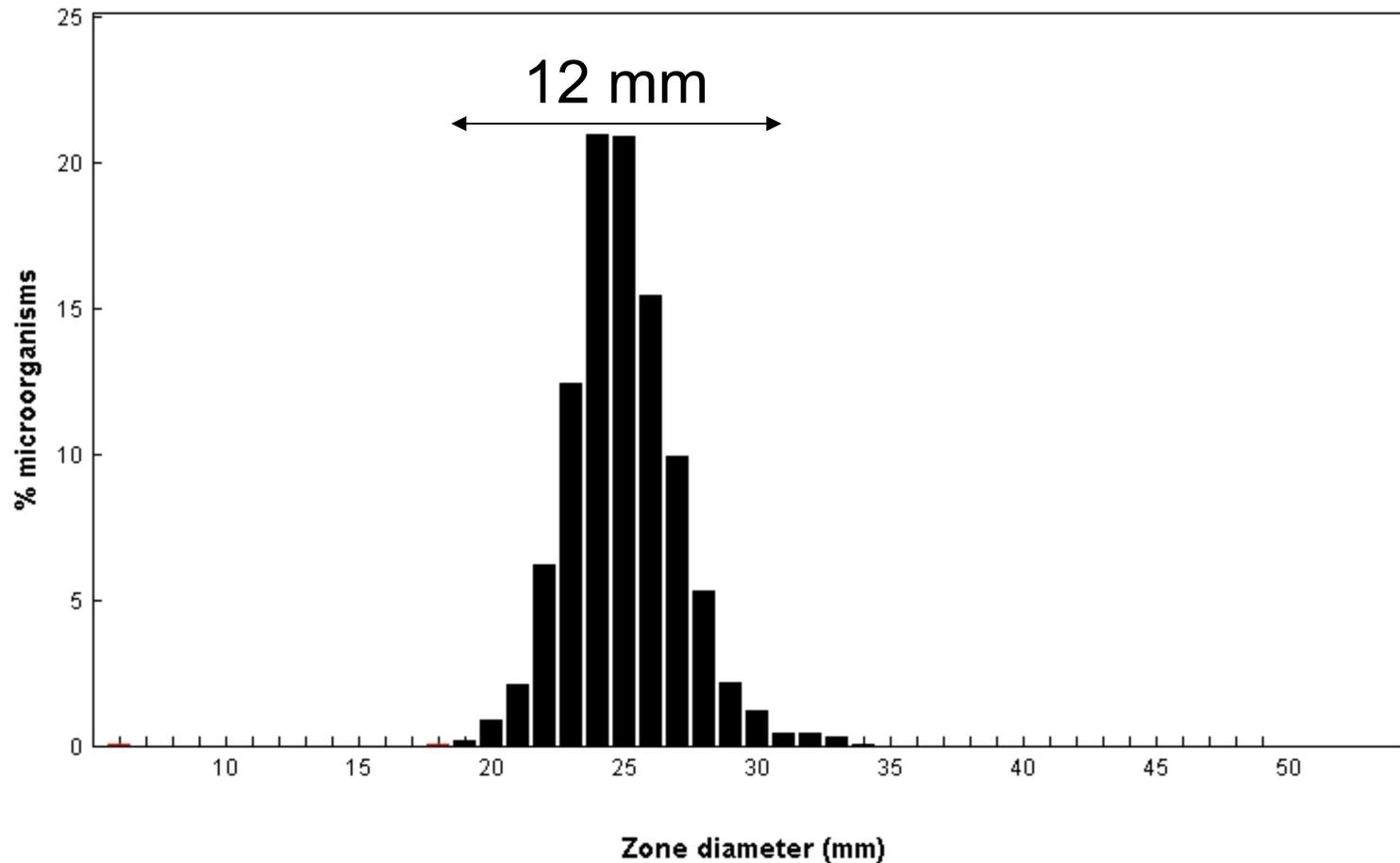
Epidemiological cut-off: WT ≤ 4 mg/L

62420 observations (22 data sources)

Clinical breakpoints: S ≤ 4 mg/L, R > 4 mg/L

**Linezolid / *Staphylococcus aureus***  
**EUCAST zone diameter distribution - Reference database 2011-05-06**  
**EUCAST disk diffusion method**

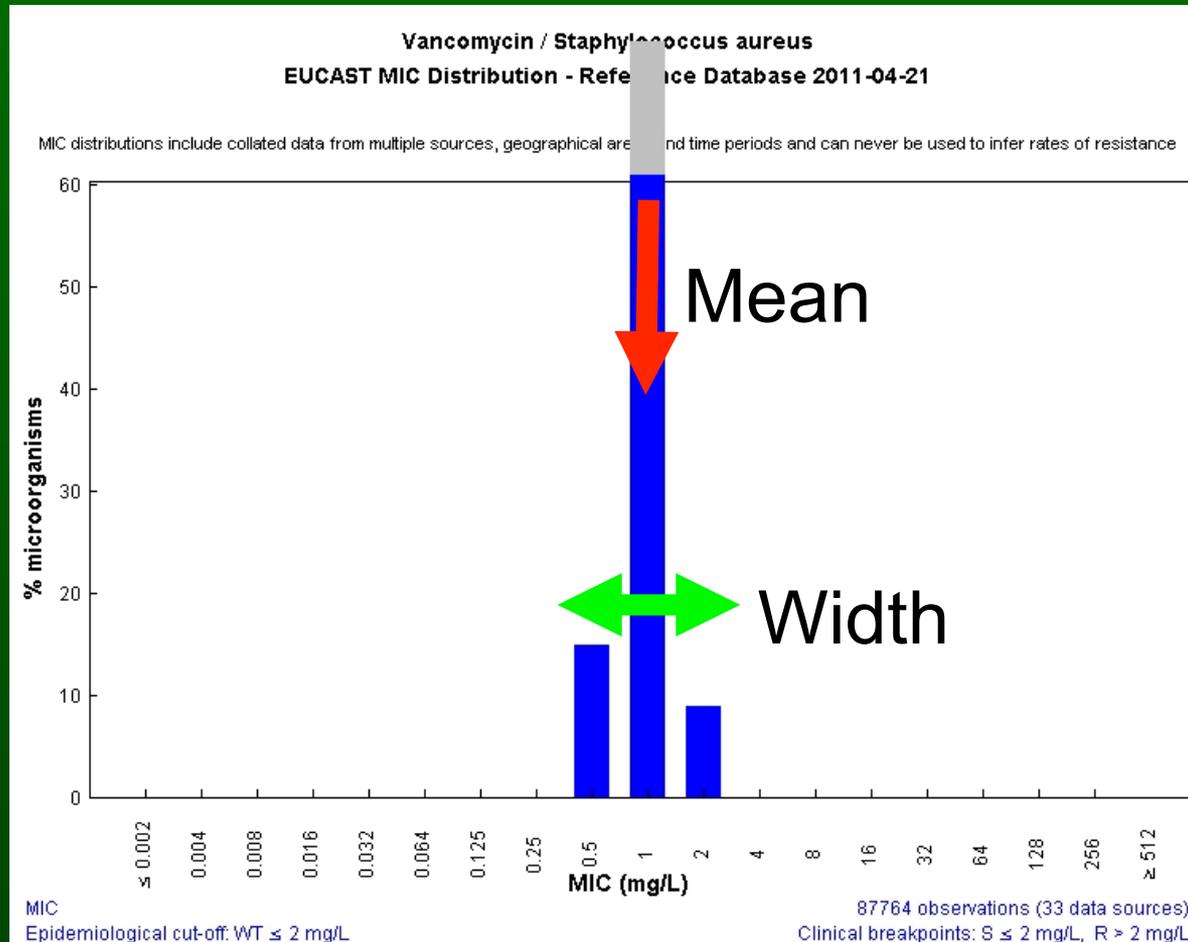
Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 10  
Epidemiological cut-off: WT  $\geq$  19 mm (MIC:  $\leq$  4 mg/L)

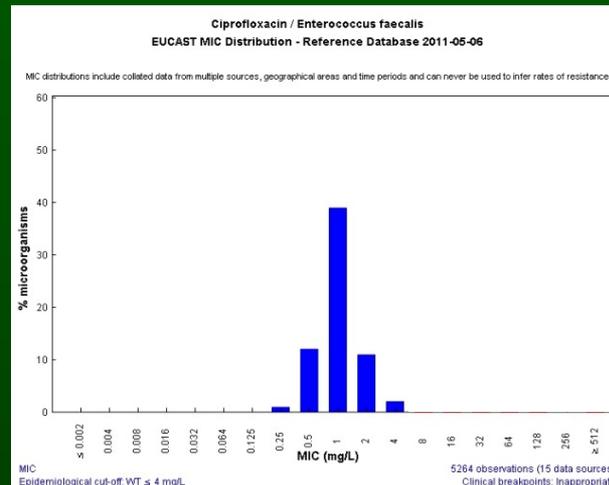
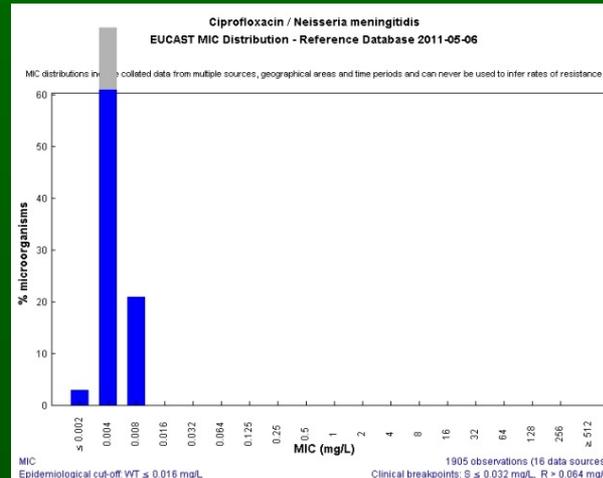
5273 observations (5 data sources)  
Clinical breakpoints: S  $\geq$  19 mm, R  $<$  19 mm

# Factors influencing the mean and width of WT distributions



# The mean of the wild type distribution

## 1. The inherent susceptibility of the species to the drug



# The mean of the wild type distribution

2. Anything **systematically** influencing the activity of the drug:

Medium – variation in MICs depending on medium

Inoculum – increasing MICs with higher inocula

Incubation – increasing MICs with longer incubation

Atmosphere – affects the activity of some drugs

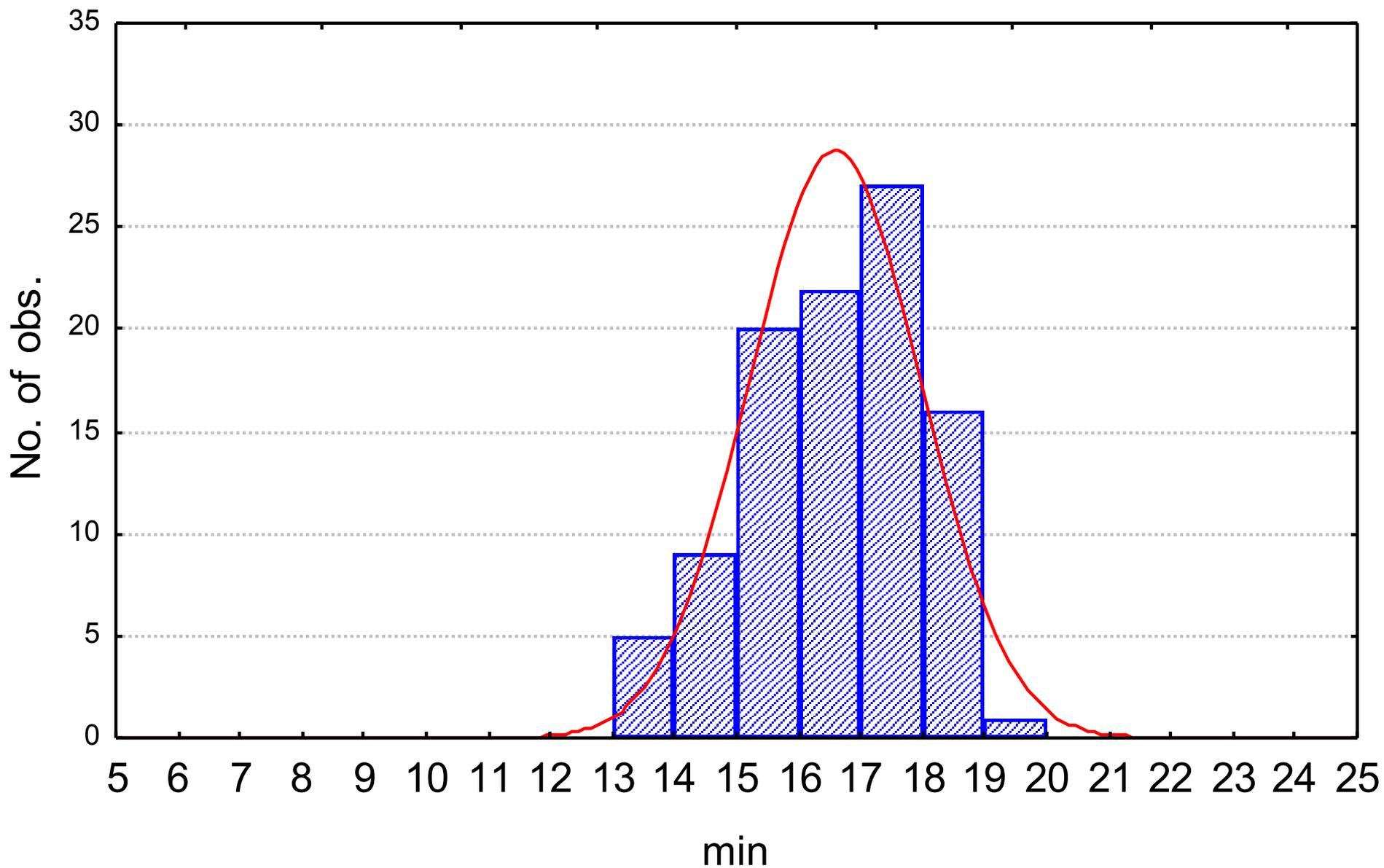
pH – some drugs are more active at high pH, others at low

# The width of the wild type distribution:

- Biological variation in **other traits** that influence the MIC
  - any biological characteristic such as generation time, nutrient dependency, atmosphere dependency etc
- Variation in **reading** (between days, between readers, between systems)
- The stability of the molecule
- Exogenous variation randomly influencing the activity of the drug
  - pH, cations, incubation atmosphere and time, etc
- ...

# *E. coli* wild type; generation times (min) (n=100)

— Expected Normal



# Standardisation

- If you provide the world at large with a not too complicated recipee **and** all the ingredients needed **and** then allow them 20 years to practice, it is not surprising that a

...tuna sallad is a tuna sallad is a tuna sallad...

...or an MIC is an MIC is an MIC...

- However, everyone wants to do their version of a Caesar sallad

# MIC reproducibility

A well standardised MIC method can at best  
provide MICs at

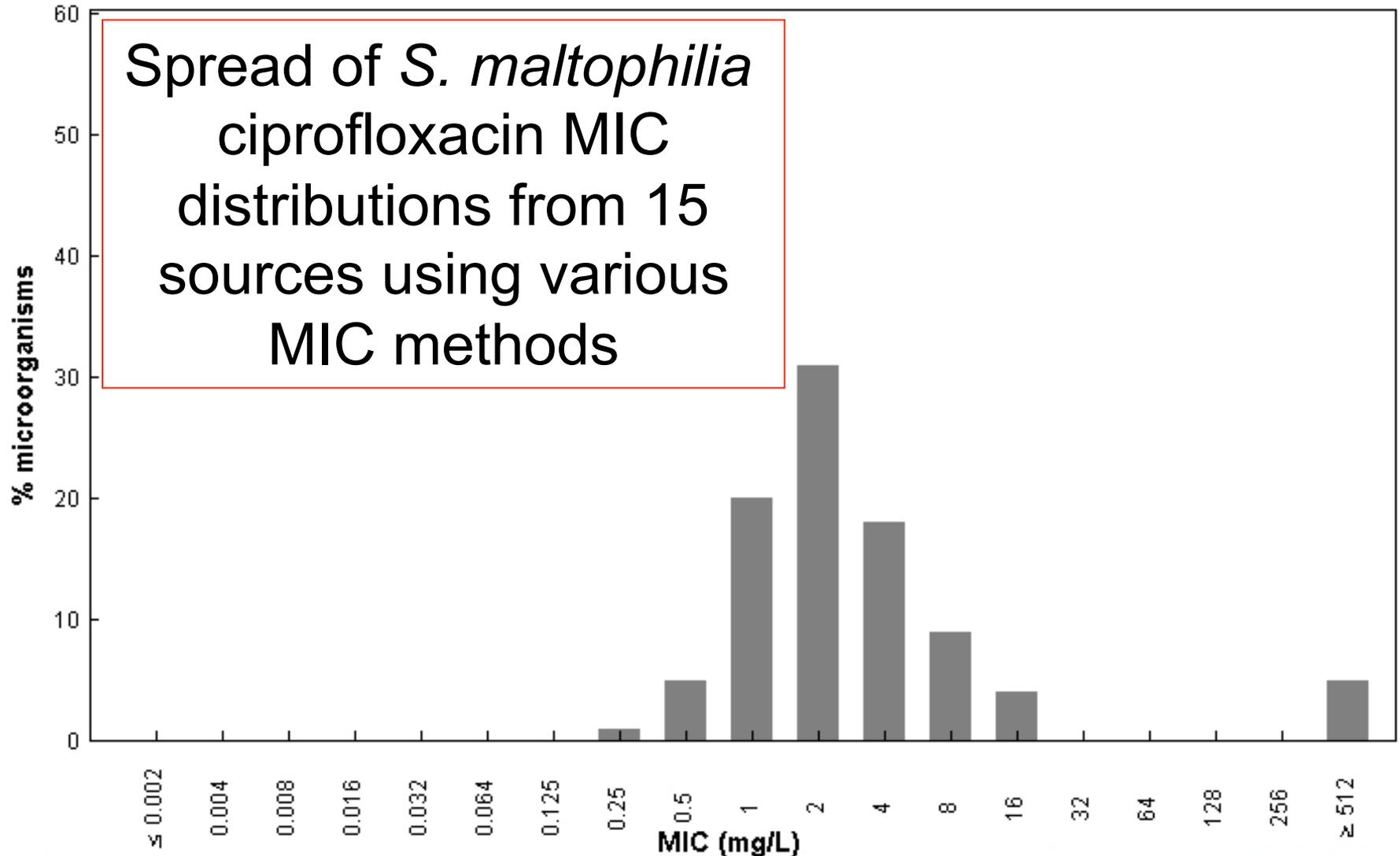
$\pm 1$  dilution step 95% of time

$\pm 2$  dilution steps 99 % of time

As a "rule of thumb" – but there are microorganisms  
and drugs with which this is not achievable

**Ciprofloxacin / *Stenotrophomonas maltophilia***  
**EUCAST MIC Distribution - Reference Database 2011-02-27**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC  
Epidemiological cut-off: -

2807 observations (15 data sources)

Clinical breakpoints: IE

# Calibration

**”MICs performed with different methods are often surprisingly similar!”**

- methods are often tightly calibrated against each other
- the degree of calibration at low end (wild type organisms) and high end (organisms with resistance mechanisms) may differ and resistance mechanisms may affect different methods to varying extent.



# A Comparison of Etest®, M.I.C.Evaluator™ Strips and CLSI Microbroth Dilution for Determining $\beta$ -lactam Antimicrobial Susceptibility in *Haemophilus influenzae*

Stephen Tristram, Human Life Sciences, University of Tasmania  
[Stephen.Tristram@utas.edu.au](mailto:Stephen.Tristram@utas.edu.au)

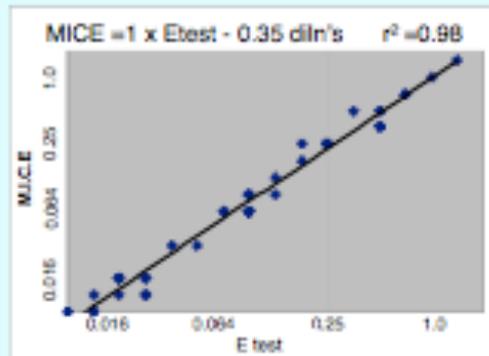


Fig 1 - M.I.C.E vs Etest for Cefotaxime MICs (mg/L)

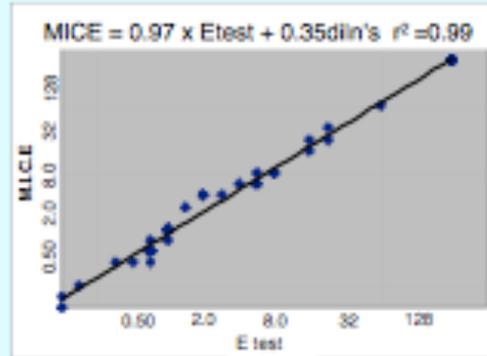


Fig 2 - M.I.C.E vs Etest for Ampicillin MICs (mg/L)

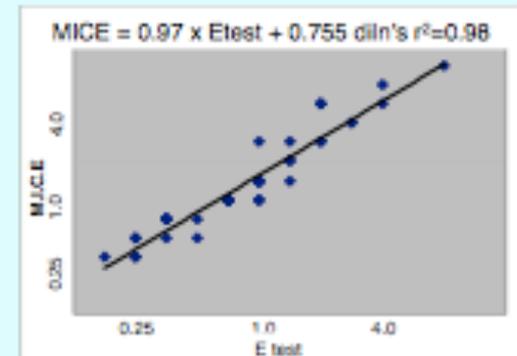


Fig 3 - M.I.C.E vs Etest for Amox-Clav MICs (mg/L)

A direct comparison between Etest and M.I.C.E showed excellent correlation for all three antibiotics (see Table 1, Figs 1, 2 and 3). There appeared to be a slight bias, with M.I.C.E strips giving MIC results approximately 0.5 doubling dilutions lower for cefotaxime and approximately 0.5 & 1.0 doubling dilutions higher for ampicillin and amox-clav respectively compared to Etest.

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# Cefalosporiner och Etest

## Kvalitetskontroll *E. coli*

### Cefotaxim resistens i Blekinge

#### Sann & Falsk

- Epidemi bland äldre
- Cefotaxim-MIC överskattades +1-1.5 sp-steg

Range 0.03-0.12

Target  
Range 0.25  
0.06-0.5

MIC on target.

MIC on upper limit.

MIC on lower limit.

MIC out of range.

# Karbapenemer och Etest

## *P. aeruginosa* kliniska isolat

### Meropenem

Isolate No	BMD MIC	Etest MIC
1	8	>32
2	8	>32
3	8	>32
4	8	>32
5	8	>32
6	8	>32
7	8	>32
8	8	>32
9	8	32
10	8	>32
11	8	>32
12	8	>32
13	4	>32
14	2	8
15	2	8
16	2	4
17	2	4
18	1	8
19	0.25	4

### Imipenem

Isolate No	BMD MIC	Etest MIC
1	8	>32
2	8	>32
3	8	>32
4	8	>32
5	4	>32
6	8	>32
7	2	4
8	2	4
9	0.25	2
10	0.5	4
11	1	1

### Doripenem

Isolate No	BMD MIC	Etest MIC
1	4	>32
2	4	32
3	4	32
4	4	>32
5	4	32
6	4	32
7	4	16
8	2	2
9	2	2

# Summarising variables that may affect MICs in broth dilution tests

- **Medium (type, brand, batch)**
  - all antibiotics and microorganisms
- **Incubation time**
  - ..the longer the higher the MIC...
- **Incubation temperature**
  - affects growth, antibiotic activity, expression of resistance mechanism, diffusion
- **Inoculum – size**
  - the larger the inoculum, the higher the MIC
- **Inoculum – growth phase**
  - the condition of the organisms affects the lag phase – the longer the lag phase, the lower the MIC
- **Atmosphere**
  - examples: CO<sub>2</sub> affects pH which affects macrolides; anaerobic atmosphere results in lower metronidazole MICs for *Helicobacter pylori*
- **pH**
  - Some antibiotics are more active in alkaline and some in acid environment.
- **Ion content**
  - Aminoglycosides are affected by Ca<sup>++</sup>, Mg<sup>++</sup> and daptomycin by Ca<sup>++</sup>
- **Reading problems**
  - Variations in readers, trailing endpoints
- **Induction of resistance mechanisms**
  - Enzymatic resistance, efflux pumps

# Temperature

**Table I.** Susceptibilities of isolates of *S. maltophilia* at 30°C and 37°C (MICs are given in mg/mL)

Antibiotic	30°C		37°C		MIC range	
	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	30°C	37°C
Gentamicin	> 128	> 128	2	64	8-> 128	0.25-128
Tobramycin	> 128	> 128	8	64	32-> 128	1-128
Amikacin	> 128	> 128	8	128	32-> 128	4-128
Polymixin B	1	4	0.5	4	0.25-64	0.25-64
Colistin	2	8	2	4	0.25-64	0.25-64
Chloramphenicol	16	16	8	16	4-64	4-32
Ceftazidime	2	64	2	64	1-64	0.25-64
Piperacillin	16	> 128	16	32	4-> 128	4-64

*Journal of Antimicrobial Chemotherapy* (1995) **36**, 317-326

**Growth temperature-dependent variation of cell envelope lipids and antibiotic susceptibility in *Stenotrophomonas (Xanthomonas) maltophilia***

A. Rahmati-Bahram, J. T. Magee and S. K. Jackson\*

# Effect of pH on antimicrobial activity

Increased activity	
Acid	Alkaline
Amoxicillin Ampicillin Piperacillin	Aminoglycosides
Tetracyclines	Erythromycin
Nitrofurantoin	Quinolones

# MIC – in summary

## Pros

- Basis for all phenotypic susceptibility testing of bacteria and fungi
- Quantifiable
- Predicts susceptibility and resistance
  - Through breakpoints – clinical resistance
  - Through ECOFFs - microbiological resistance
- Can be highly standardized for most antibiotics and many species
- "End result"

## Cons

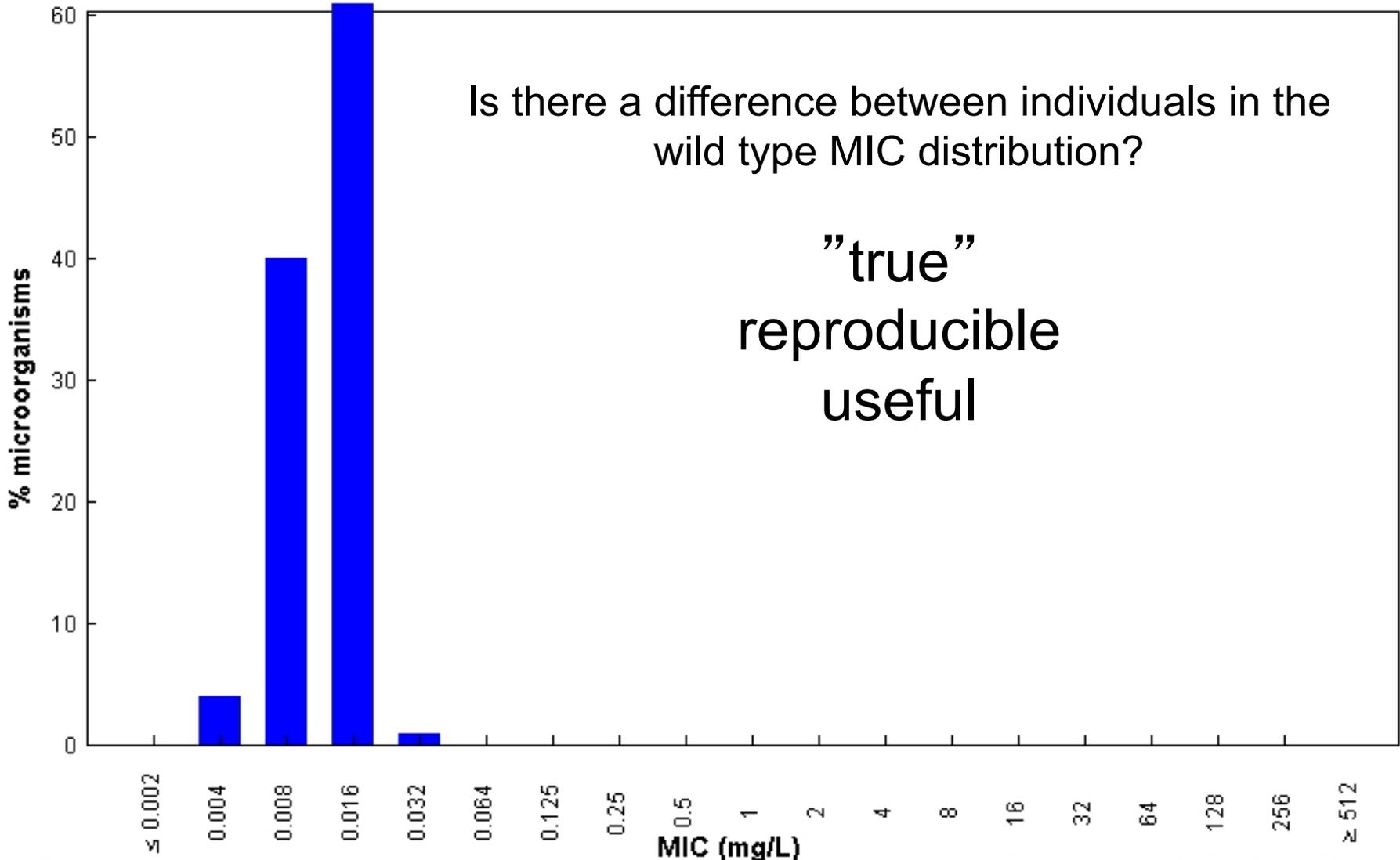
- In vitro
- Relative - accuracy dependant on agreement
- Discontinuous variable
- Reproducibility +/- 1 dilution step.
- Requires high degree of standardisation
- Methods for fastidious organisms still poorly standardized – "lots of individual fixes to make it work"
- "End result"

The End

Thank you

# An MIC is an MIC is an MIC, isn't it?

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC

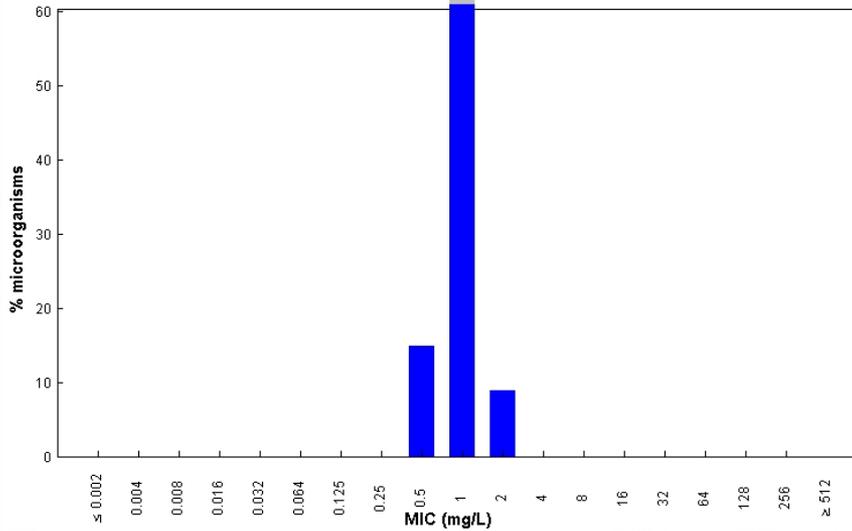
Epidemiological cut-off: WT ≤ 0.064 mg/L

3615 observations (11 data sources)

Clinical breakpoints: S ≤ 0.25 mg/L, R > 0.25 mg/L

**Vancomycin / *Staphylococcus aureus***  
**EUCAST MIC Distribution - Reference Database 2011-04-21**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC 87764 observations (33 data sources)  
 Epidemiological cut-off: WT ≤ 2 mg/L Clinical breakpoints: S ≤ 2 mg/L, R > 2 mg/L

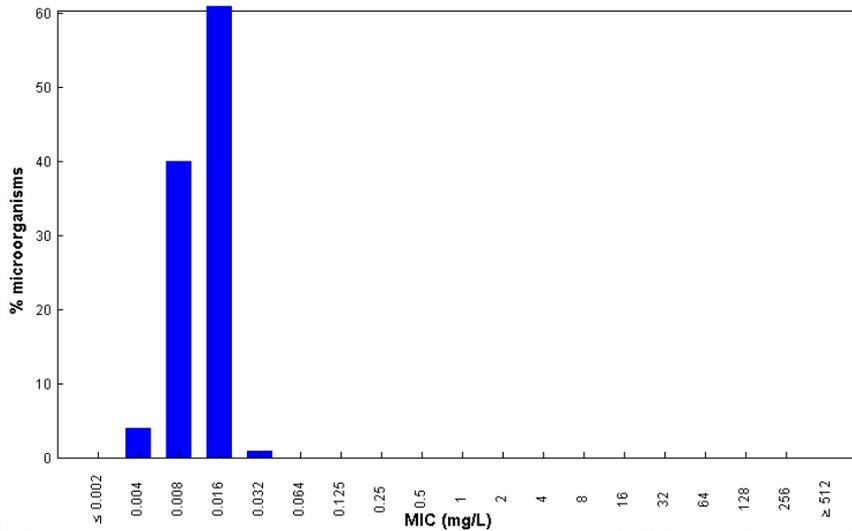
3

4

5

**Benzylpenicillin / *Streptococcus pyogenes***  
**EUCAST MIC Distribution - Reference Database**

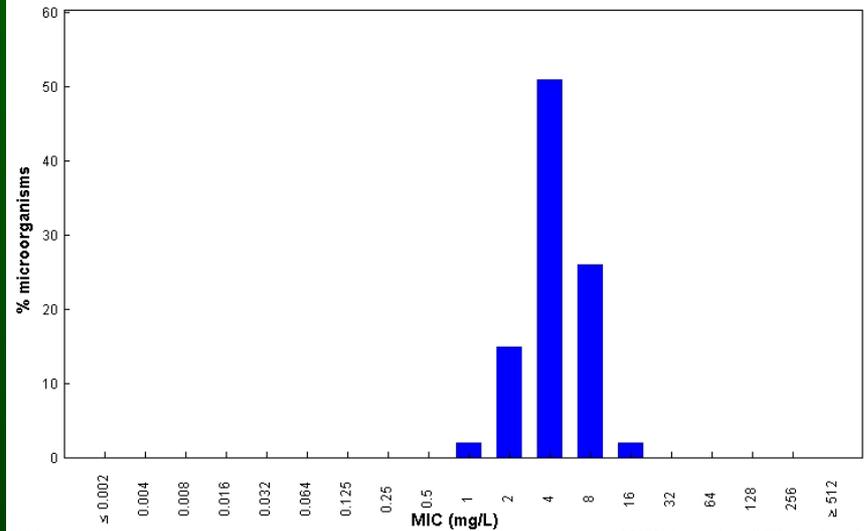
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC 3615 observations (11 data sources)  
 Epidemiological cut-off: WT ≤ 0.064 mg/L Clinical breakpoints: S ≤ 0.25 mg/L, R > 0.25 mg/L

**Erythromycin / *Haemophilus influenzae***  
**EUCAST MIC Distribution - Reference Database 2011-04-25**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



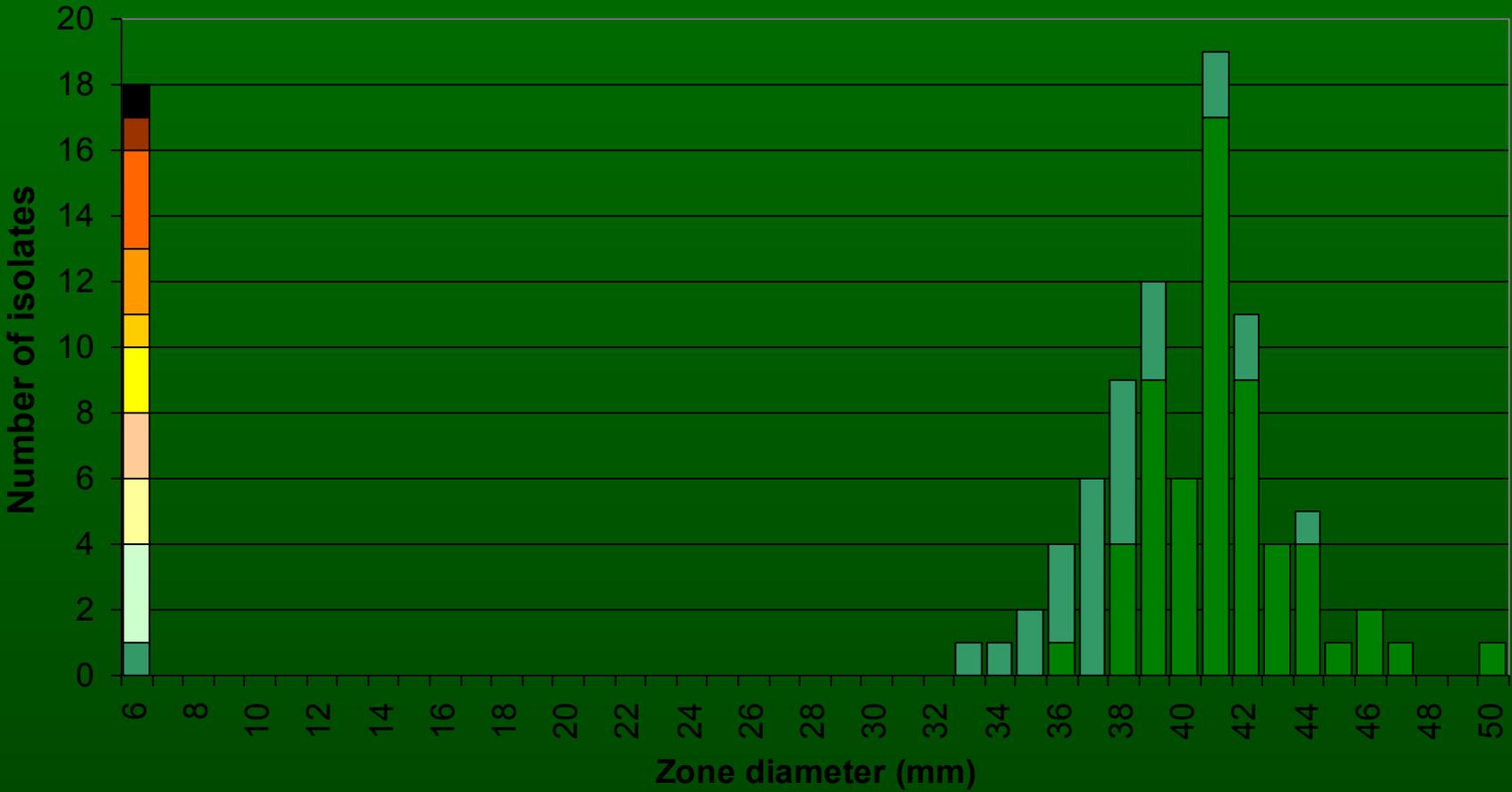
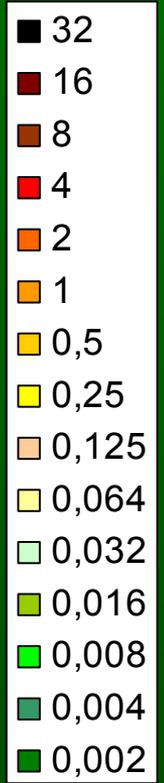
MIC 29049 observations (13 data sources)  
 Epidemiological cut-off: WT ≤ 16 mg/L Clinical breakpoints: S ≤ 0.5 mg/L, R > 16 mg/L

# Neisseria gonorrhoeae

## Zone diameters on Nalidixic acid 30 µg

### Ciprofloxacin MICs

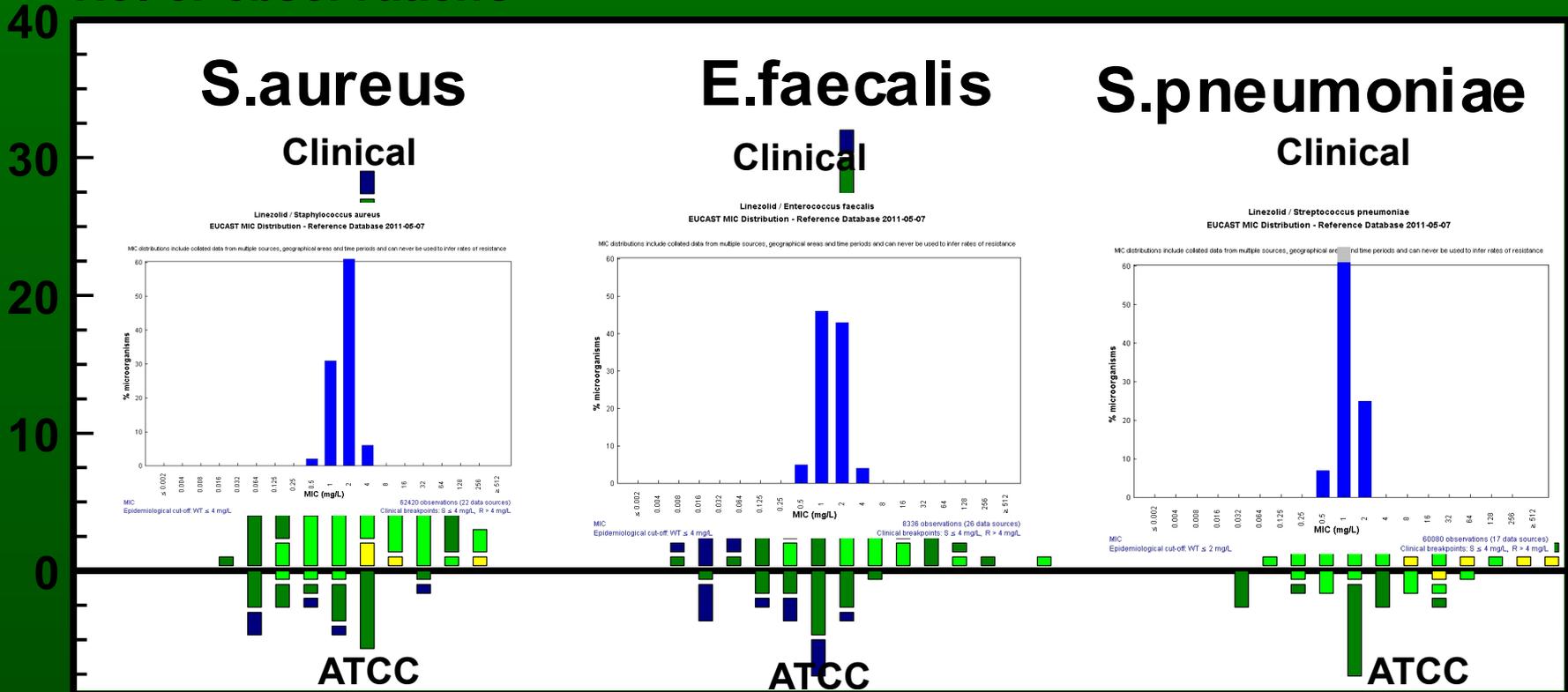
Ciprofloxacin MIC



Data by Hanna Odén, Clinical microbiology, Växjö, Sweden

# MIC distributions in zone diameter histograms Linezolid

No. of observations



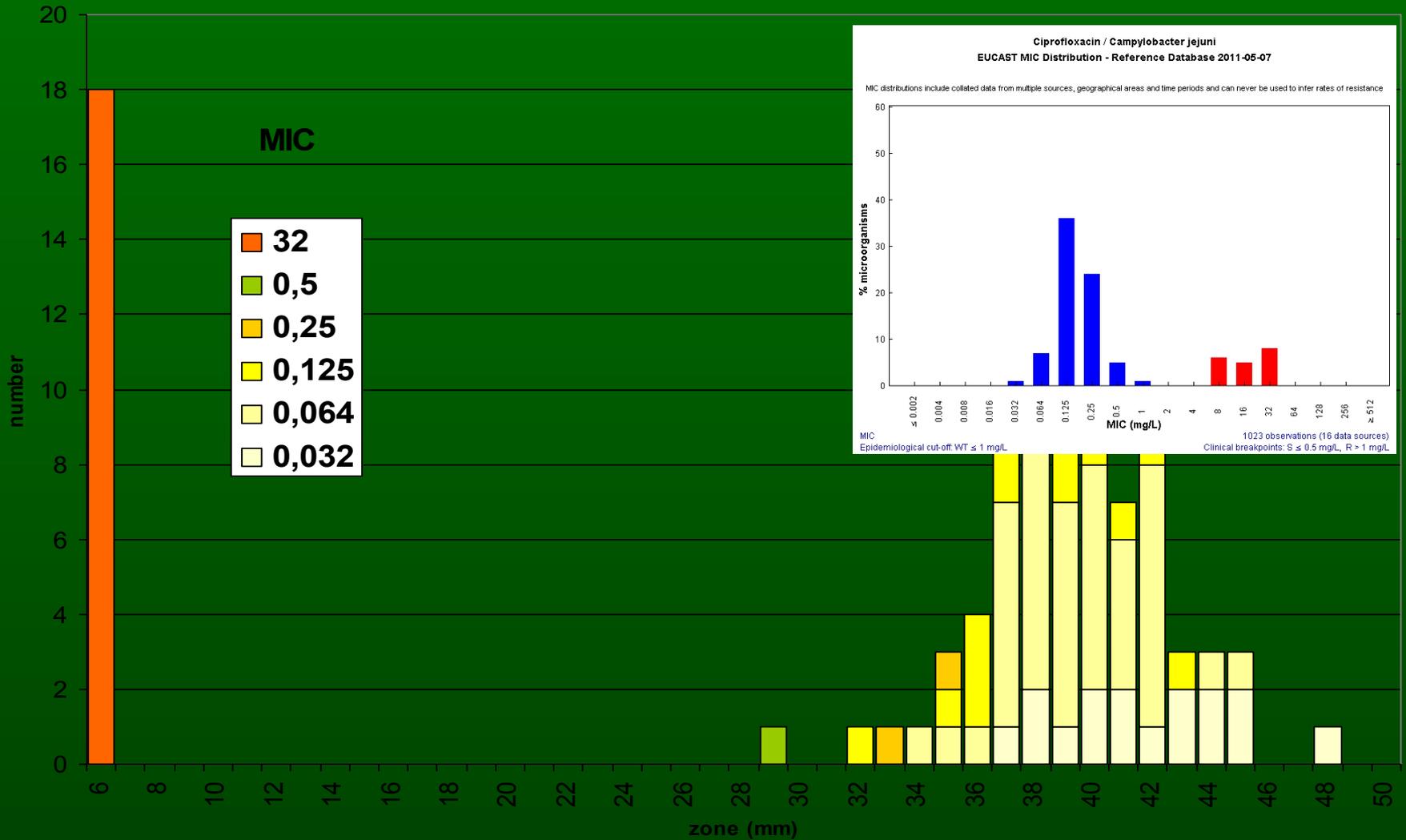
20 22 24 26 28 30 32 34  
21 23 25 27 29 31 33 35

Inhibition zone (mm)



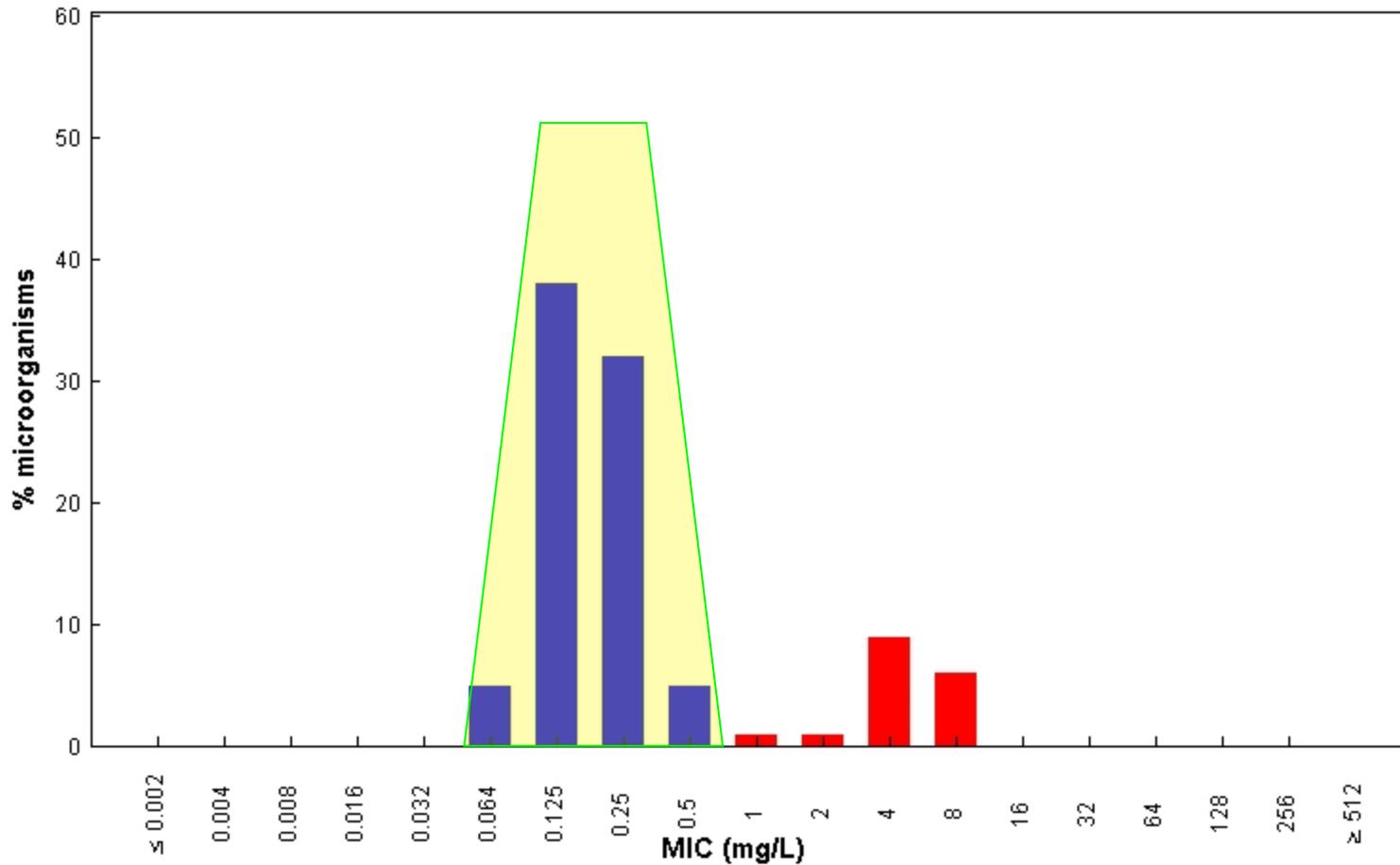
# Campylobacter

## Ciprofloxacin 5 µg disc vs. Ciprofloxacin MICs



**Levofloxacin / *Staphylococcus aureus***  
**EUCAST MIC Distribution - Reference Database 2011-05-06**

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC  
Epidemiological cut-off: WT ≤ 0.5 mg/L

27556 observations (13 data sources)  
Clinical breakpoints: S ≤ 1 mg/L, R > 2 mg/L

