



Pågående projekt  
EUCAST  
lappdiffusionsmetod

NordicAST Workshop 2013

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# Pågående projekt

- Ceftaroline, brytpunkter och QC-kriterier
- *S. pseudintermedius* och cefoxitin
- *Pseudomonas non-aeruginosa*
- Lappdiffusion anaerober
- Amoxicillin-klavulansyra och ampicillin-sulbaktam
- MIC-gränser för *H. influenzae* NCTC 8468

# Ceftarolin

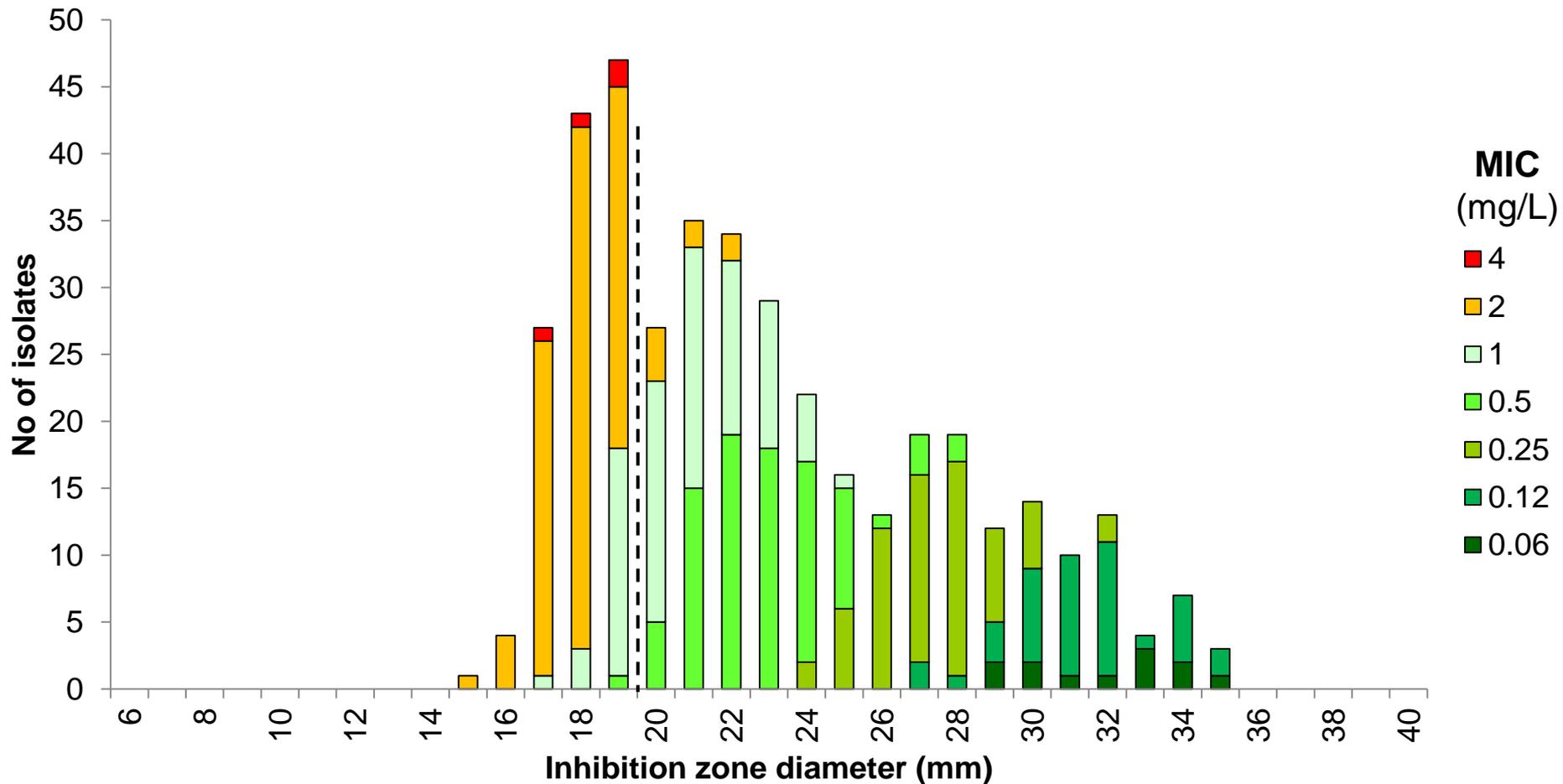
- Ny cefalosporin, Astra Zeneca
- Indikationer:
  - Komplicerade infektioner i hud och mjukdelar (cSSTI)
  - Samhällsförvärvad pneumoniae (CAP)
- Effekt på många MRSA ( $MIC \leq 1$  mg/l) och pneumokocker med nedsatt känslighet för penicilliner.

# Ceftarolin

- Samarbete med LSI (Laboratory Specialists Inc., USA)
- Lappstyrka
  - Ceftaroline 5, 10 och 30 µg → 5 µg
- Kliniska isolat
  - 187 Enterobacteriaceae (109 *E. coli*, 78 övriga)
  - 100 *S. aureus* (30 MSSA, 70 MRSA)
- Zon/MIC-korrelat för ceftarolin
  - EUCAST: Lappdiffusion
  - LSI: Lappdiffusion och MIC med buljongspädning

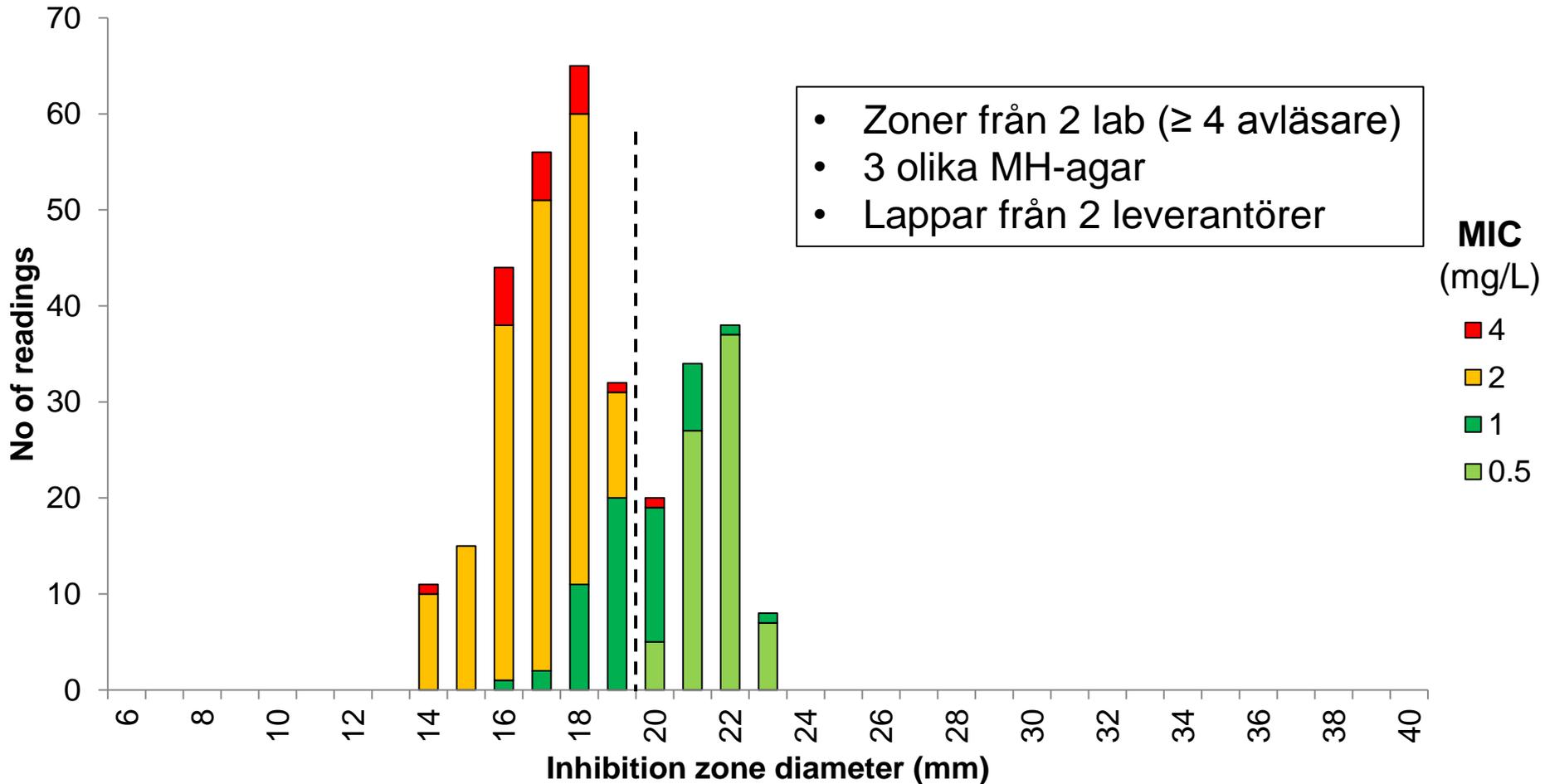
# Ceftaroline 5 µg vs. MIC

## *S. aureus*, 100 clinical isolates tested at 2 sites x2



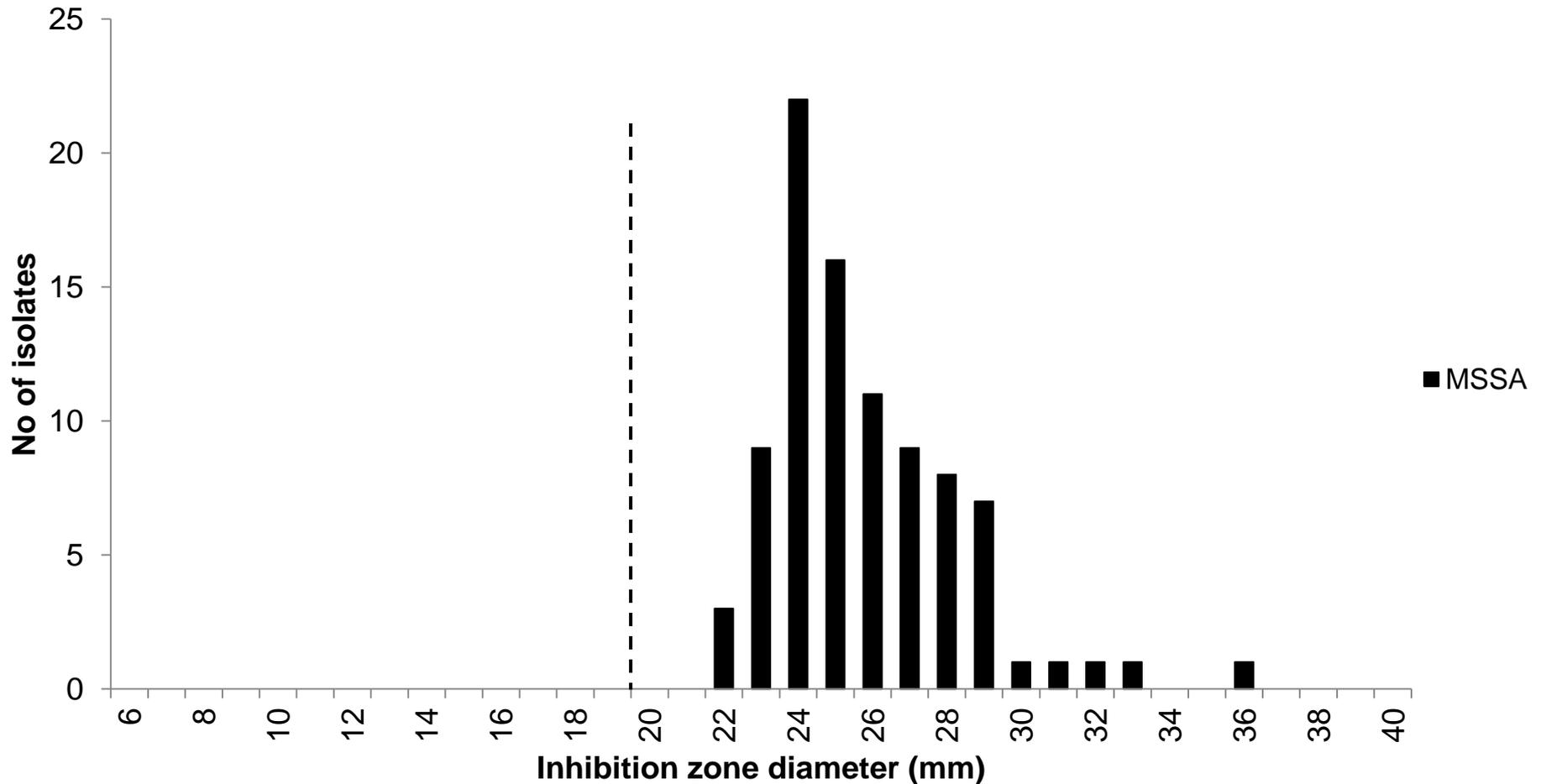
|                    |                        |                    |
|--------------------|------------------------|--------------------|
| <b>Brytpunkter</b> |                        | <b>ECOFF</b>       |
| MIC                | $S \leq 1, R > 1$ mg/L | $WT \leq 0.5$ mg/L |
| Zondiameter        | $S \geq 20, R < 20$ mm |                    |

***S. aureus* vs. Ceftaroline 5 µg (Oxoid and MAST disks)**  
**17 selected isolates tested x15 (N=323)**



|                    |                        |                    |
|--------------------|------------------------|--------------------|
| <b>Brytpunkter</b> |                        | <b>ECOFF</b>       |
| MIC                | $S \leq 1, R > 1$ mg/L | $WT \leq 0.5$ mg/L |
| Zondiameter        | $S \geq 20, R < 20$ mm |                    |

# Ceftaroline 5 µg vs. mecA status MSSA, 90 clinical isolates



## Brytpunkter

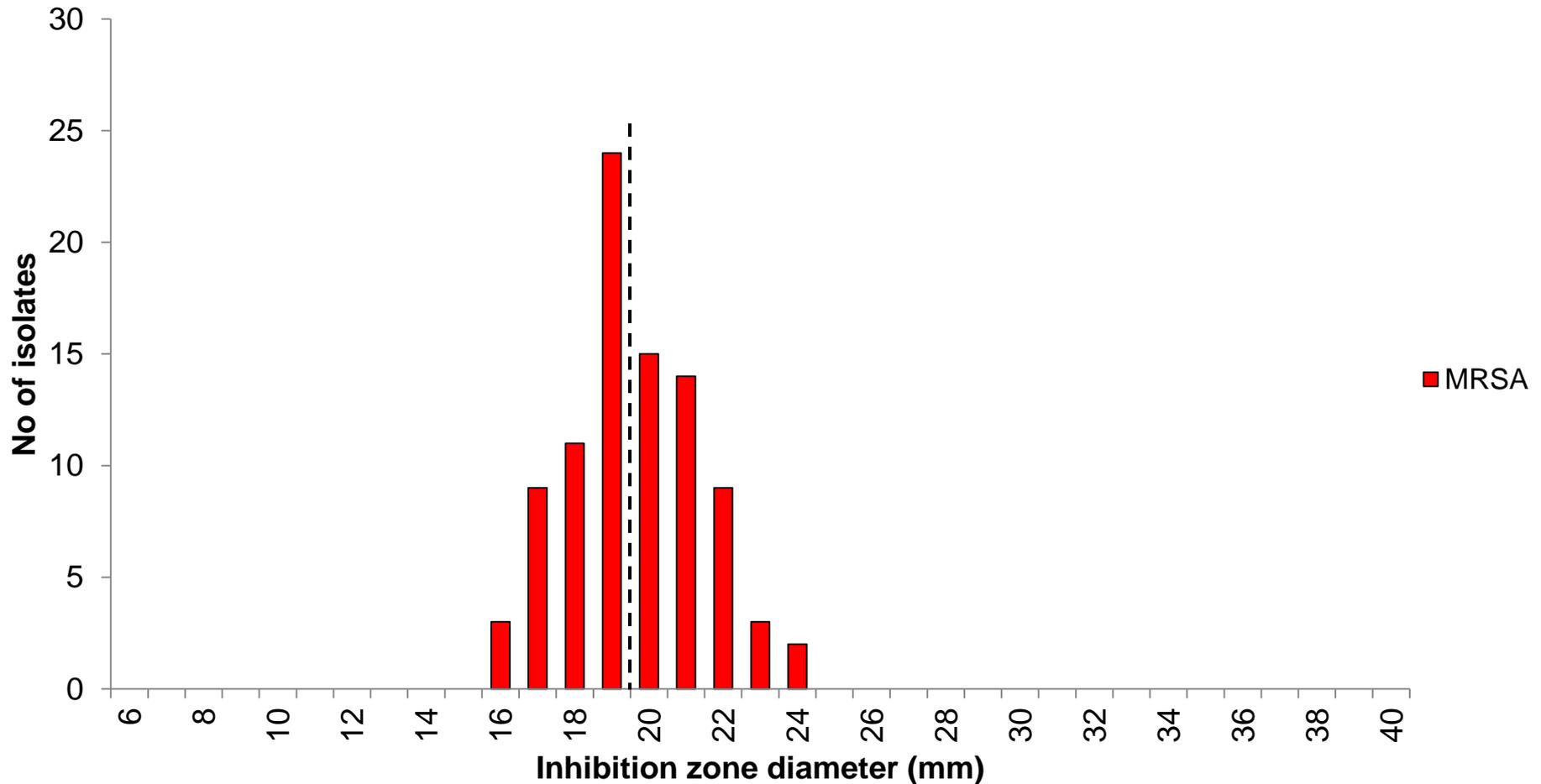
MIC  $S \leq 1$ ,  $R > 1$  mg/L

Zondiameter  $S \geq 20$ ,  $R < 20$  mm

## ECOFF

WT  $\leq 0.5$  mg/L

# Ceftaroline 5 µg vs. *mecA* status MRSA, 90 clinical isolates



## Brytpunkter

MIC S ≤ 1, R > 1 mg/L

Zondiameter S ≥ 20, R < 20 mm

## ECOFF

WT ≤ 0.5 mg/L

# Ceftarolin brytpunkter

| Organisms  | MIC breakpoint (mg/L) |                   | Disk content (µg) | Zone diameter breakpoint (mm) |                   |
|--|-----------------------|-------------------|-------------------|-------------------------------|-------------------|
|  | S ≤                   | R >               |                   | S ≥                           | R <               |
| Enterobacteriaceae                                   | 0.5                   | 0.5               |                   | 23                            | 23                |
| <i>Staphylococcus aureus</i>                         | 1                     | 1                 | 5                 | 20 <sup>1</sup>               | 20 <sup>1</sup>   |
| Streptococcus Groups A, B, C, G                      | Note <sup>2</sup>     | Note <sup>2</sup> |                   | Note <sup>2</sup>             | Note <sup>2</sup> |
| <i>Streptococcus pneumoniae</i>                      | 0.25                  | 0.25              |                   | Note <sup>3</sup>             | Note <sup>3</sup> |
| <i>Haemophilus influenzae</i>                        | 0.03                  | 0.03              |                   | Note <sup>4</sup>             | Note <sup>4</sup> |
| Pk/Pd (non-species related) breakpoints <sup>2</sup> | 0.5                   | 0.5               |                   | -                             | -                 |

<sup>1</sup> Methicillin-susceptible isolates can be reported susceptible to ceftaroline without further testing. For methicillin-resistant isolates with ceftaroline zone diameters 19-21 mm, determine the MIC to confirm the susceptibility.

<sup>2</sup> Infer from the benzylpenicillin susceptibility (MIC or zone diameter)

<sup>3</sup> Infer from the oxacillin 1 µg disk screen

<sup>4</sup> Infer from the benzylpenicillin 1 unit disk screen

# Ceftarolin QC-gränser

- Ceftaroline 5 µg

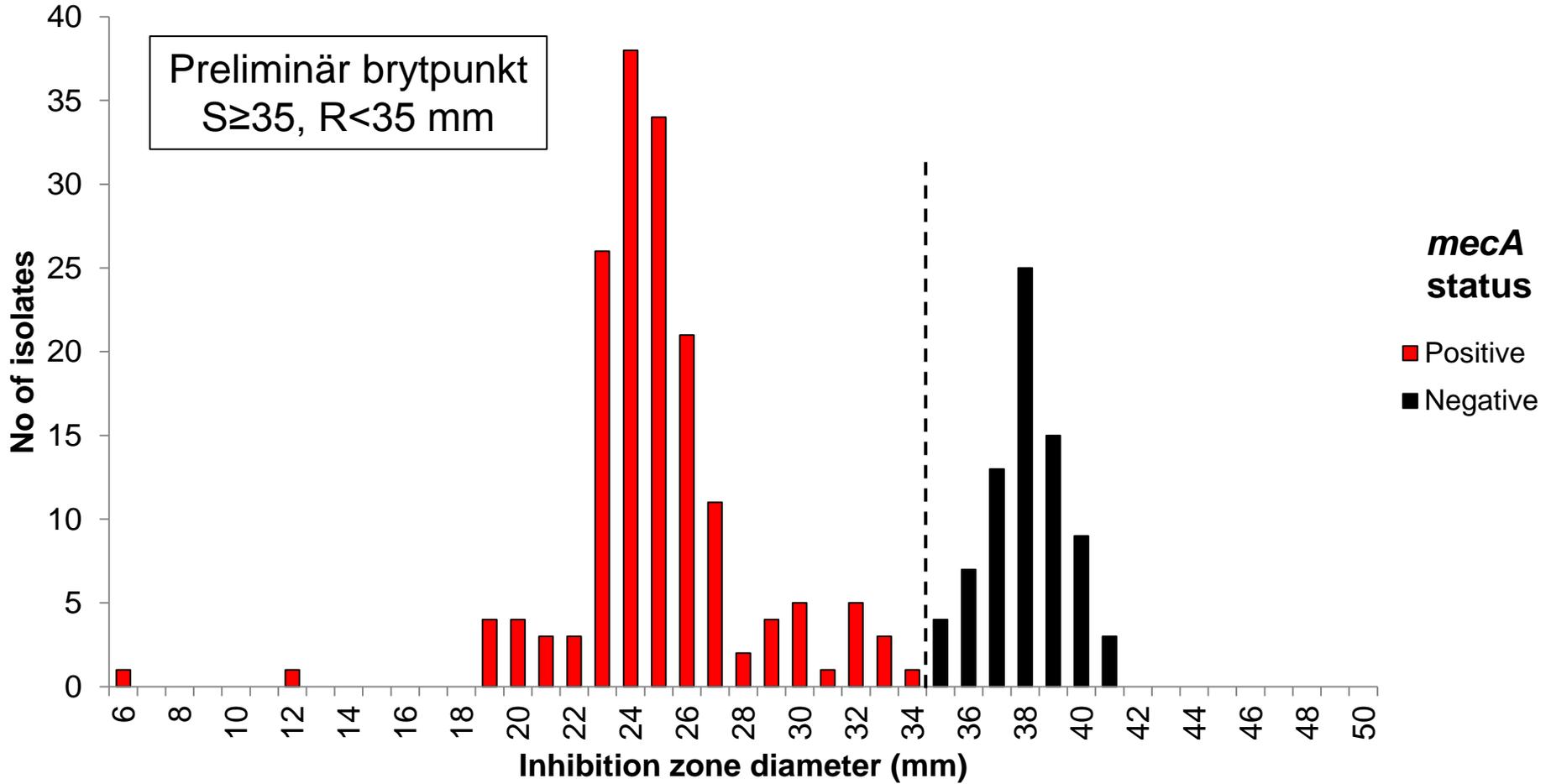
| <b>Kontrollstam</b>         | <b>Range<br/>(mm)</b> | <b>Target<br/>(mm)</b> |
|-----------------------------|-----------------------|------------------------|
| <i>E. coli</i> ATCC 25922   | 24-30                 | 27                     |
| <i>S. aureus</i> ATCC 29213 | 24-30                 | 27                     |

Screenbrytpunkt för cefoxitin  
30 µg och *S. pseudintermedius*

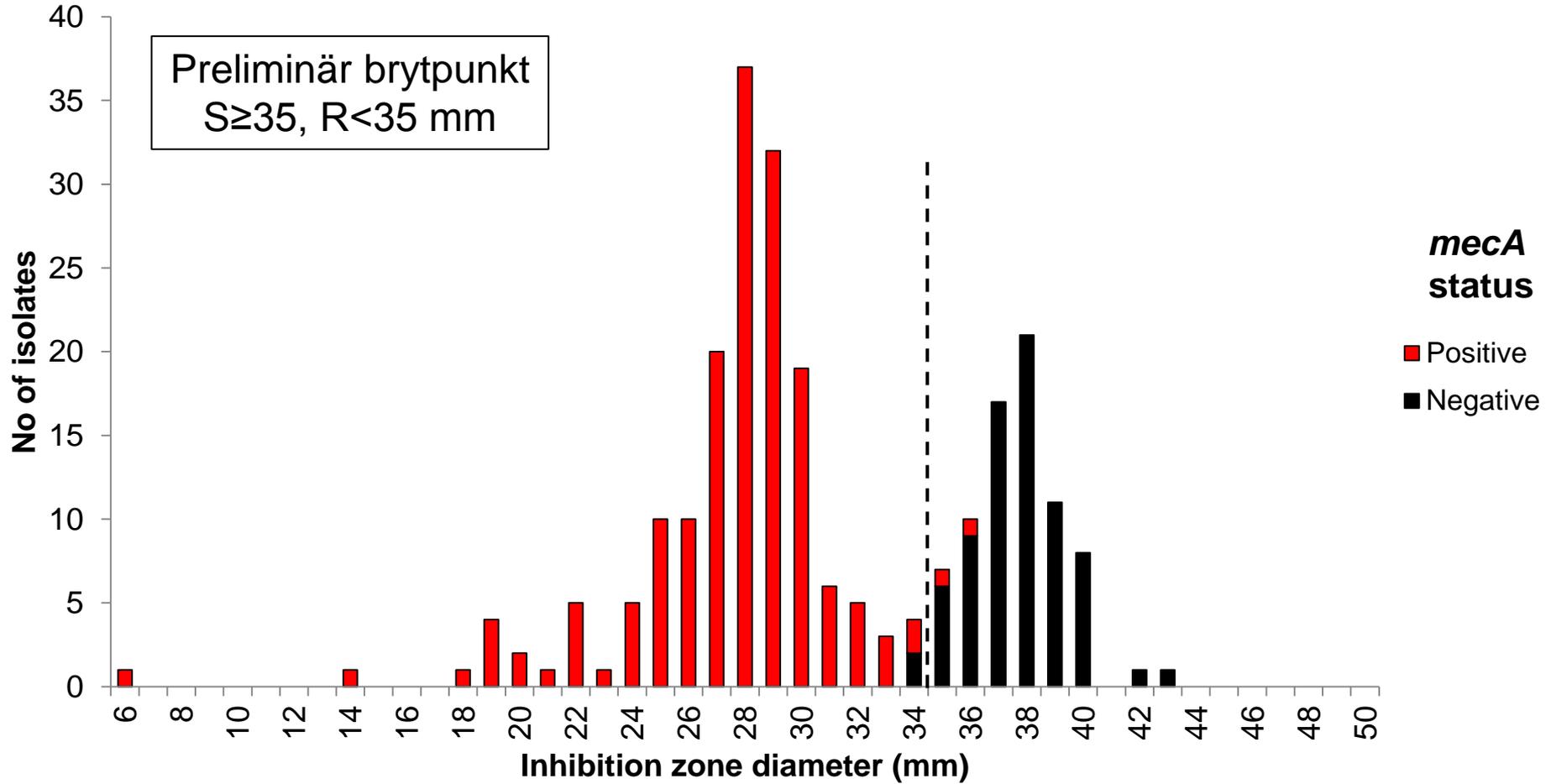
# *S. pseudintermedius*

- Koagulas-positiv stafylokokk
- Vanlig hos djur, ffa hund och katt.
- 243 isolat:
  - Statens Veterinärmedicinska anstalt
  - Veterinærinstituttet (Norge)
- Artbestämning med MALDI-TOF
- Lappdiffusion med cefoxitin 30 µg
- *mecA* enligt PCR

# Cefoxitin 30 µg vs. *mecA* status on BBL MH *S. pseudintermedius*, 243 clinical isolates

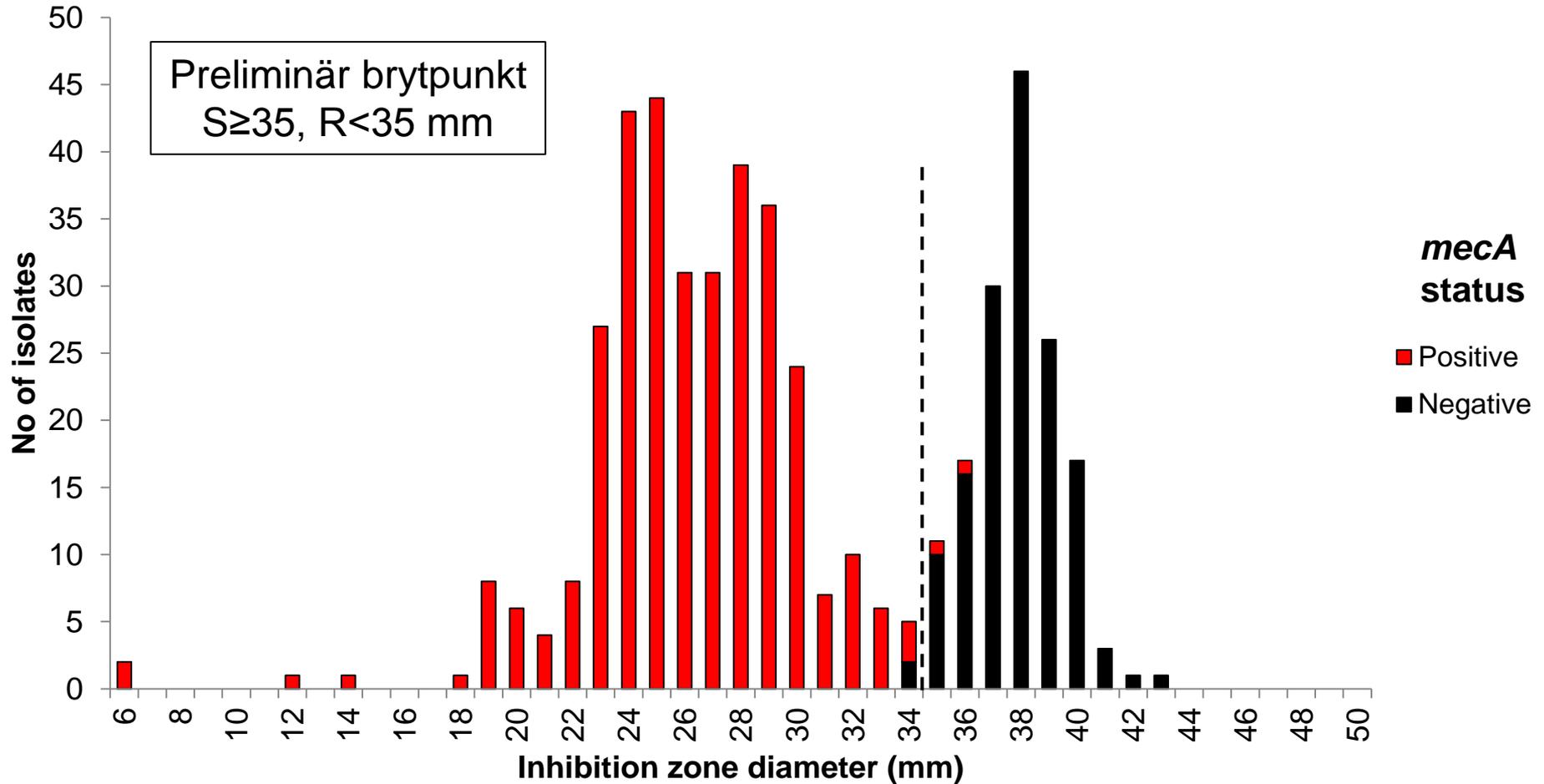


# Cefoxitin 30 $\mu$ g vs. *mecA* status on Oxoid MH *S. pseudintermedius*, 243 clinical isolates



# Cefoxitin 30 µg vs. *mecA* status

*S. pseudintermedius*, 243 clinical isolates tested in duplicate



*Pseudomonas non-aeruginosa*

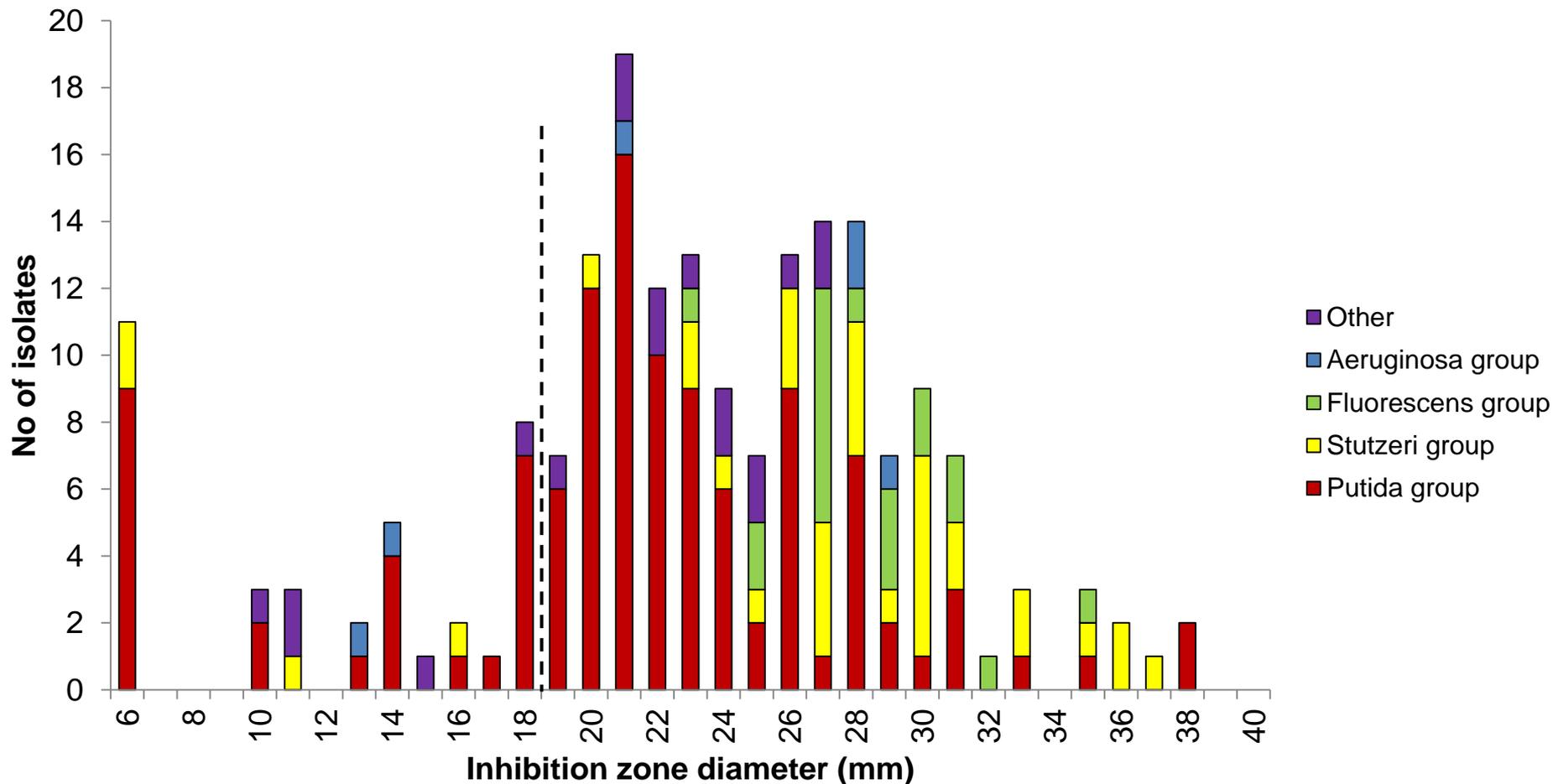
# *Pseudomonas non-aeruginosa*

- 192 kliniska isolat från SENTRY (JMI, USA)
- Artidentifiering med MALDI-TOF
- Zon/MIC-korrelat
  - EUCAST: Lappdiffusion
  - JMI: MIC med buljongspädning

# *Pseudomonas non-aeruginosa*

- MALDI-TOF resultat för 192 kliniska isolat
  - Putida-gruppen: 113
    - P. putida*, *P. oryzihabitans*, *P. monteilii*, *P. mosselii*,  
*P. plecoglossicida*, *P. fulva*.
  - Stutzeri-gruppen: 35
    - P. stutzeri*, *P. luteola*, *P. balearica*
  - Flourescens-gruppen: 20
    - P. flourescens*, *P. synxantha*, *P. libanensis*, *P. azotoformans*,  
*P. extremorientalis*, *P. rhodesiae*, *P. antarctica*.
  - Aeruginosa-gruppen: 6
    - P. mendocina*, *P. oleovorans*.
  - Övriga: 18
    - P. chlororaphis*, *P. koreensis*, *P. graminis*, *P. asplenii*

## Piperacillin-tazobactam 30-6 µg vs. species 192 clinical isolates, *Pseudomonas non-aeruginosa*



### Brytpunkter

MIC S ≤ 16, R > 16 mg/L

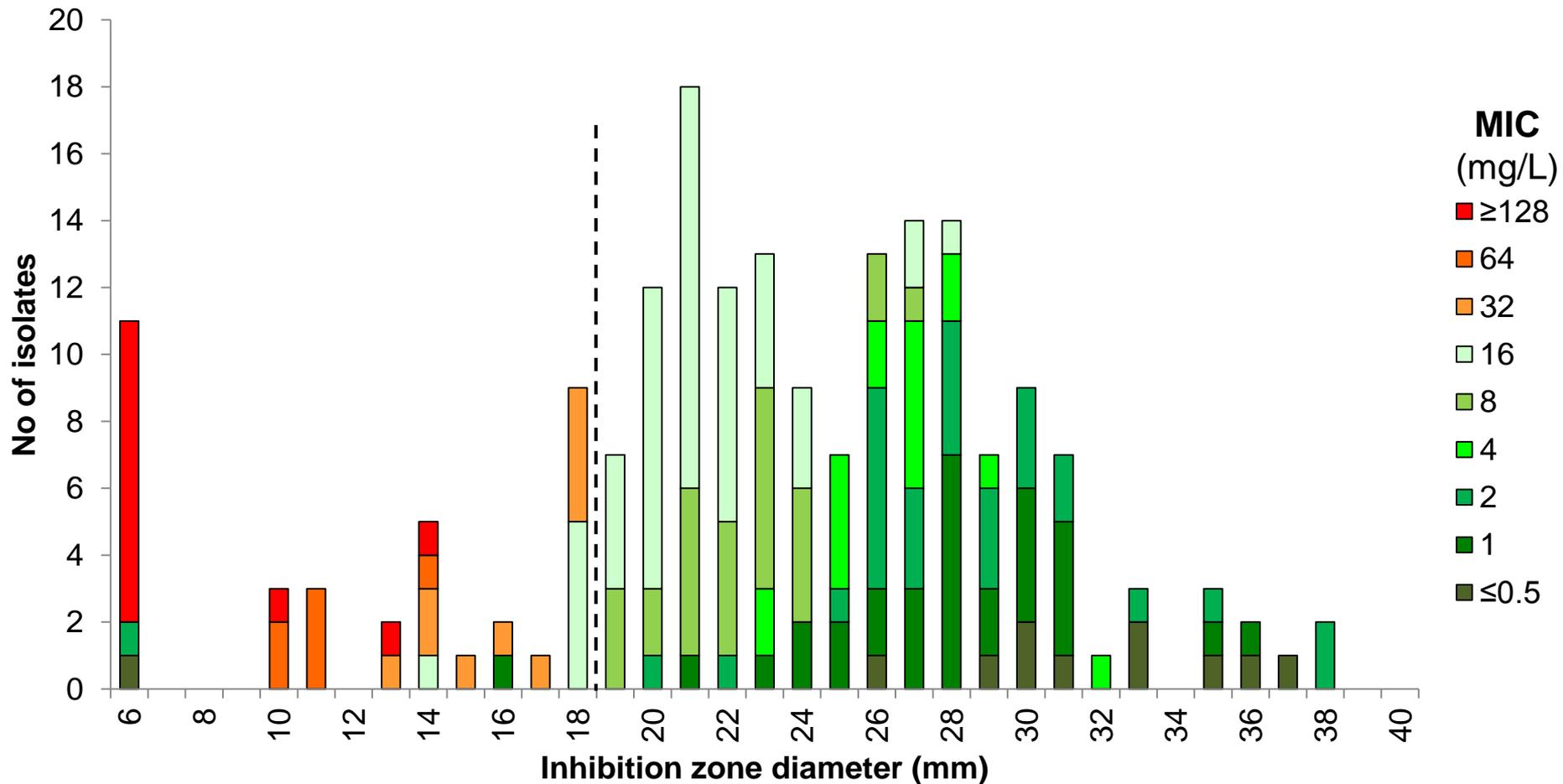
Zondiameter S ≥ 19, R < 19 mm

### ECOFF

WT ≤ 16 mg/L

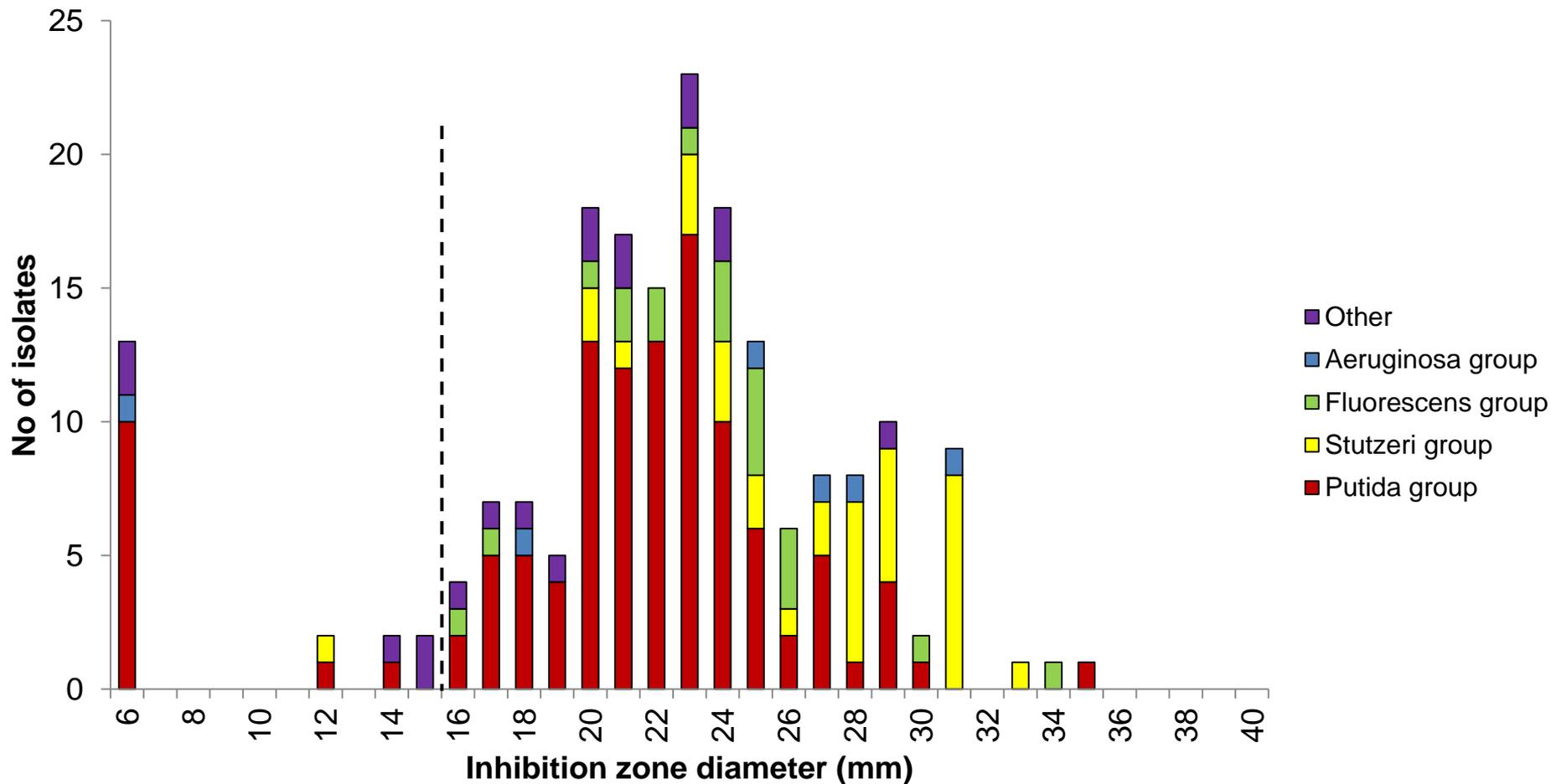
# Piperacillin-tazobactam 30-6 µg vs. MIC

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



|                    |                     |
|--------------------|---------------------|
| <b>Brytpunkter</b> | <b>ECOFF</b>        |
| MIC                | S ≤ 16, R > 16 mg/L |
| Zondiameter        | WT ≤ 16 mg/L        |
|                    | S ≥ 19, R < 19 mm   |

## Ceftazidime 10 µg vs. species 192 clinical isolates, *Pseudomonas non-aeruginosa*



### Brytpunkter

MIC

S ≤ 8, R > 8 mg/L

Zondiameter

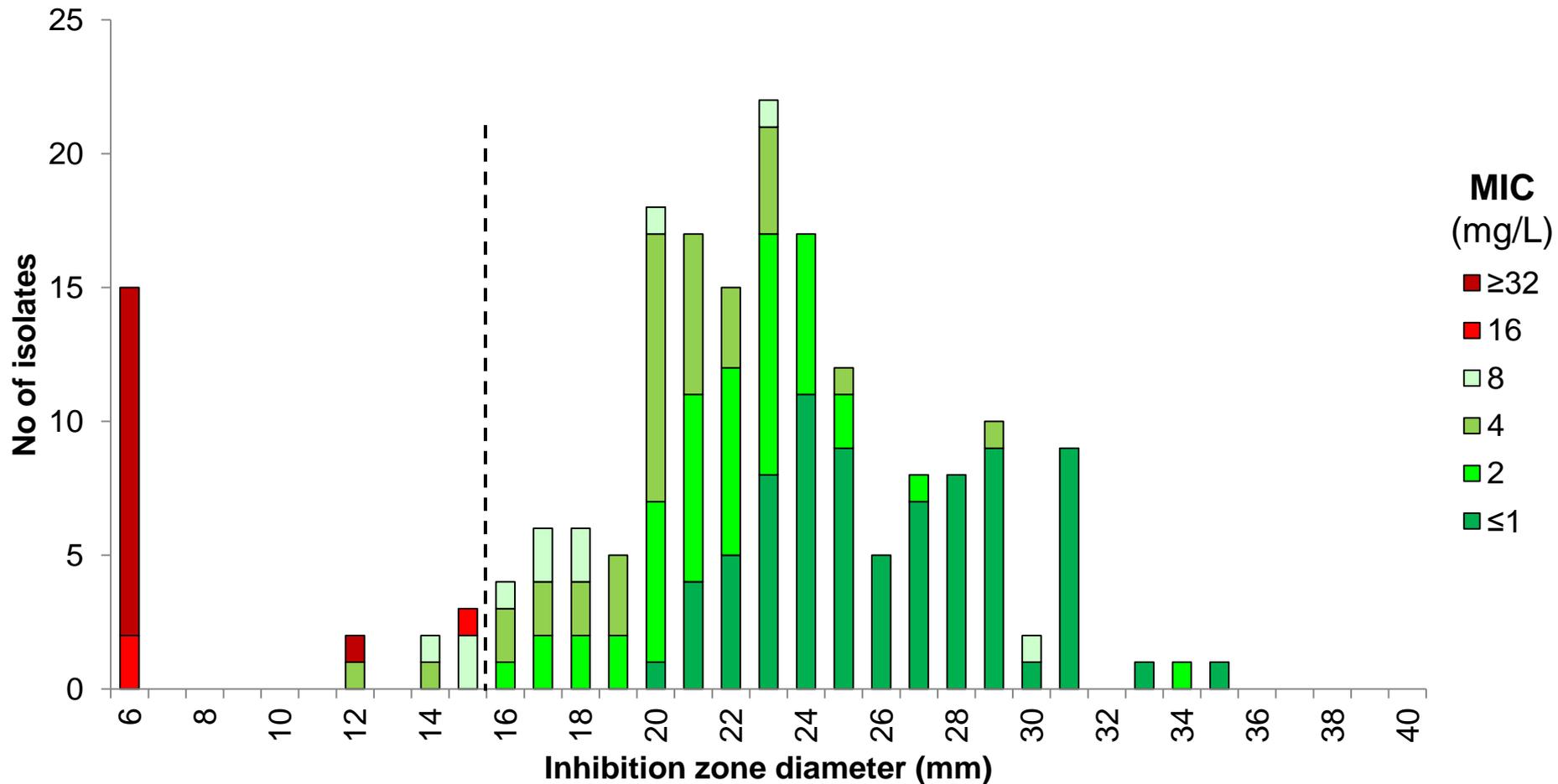
S ≥ 16, R < 16 mm

### ECOFF

WT ≤ 8 mg/L

# Ceftazidime 10 µg vs. MIC

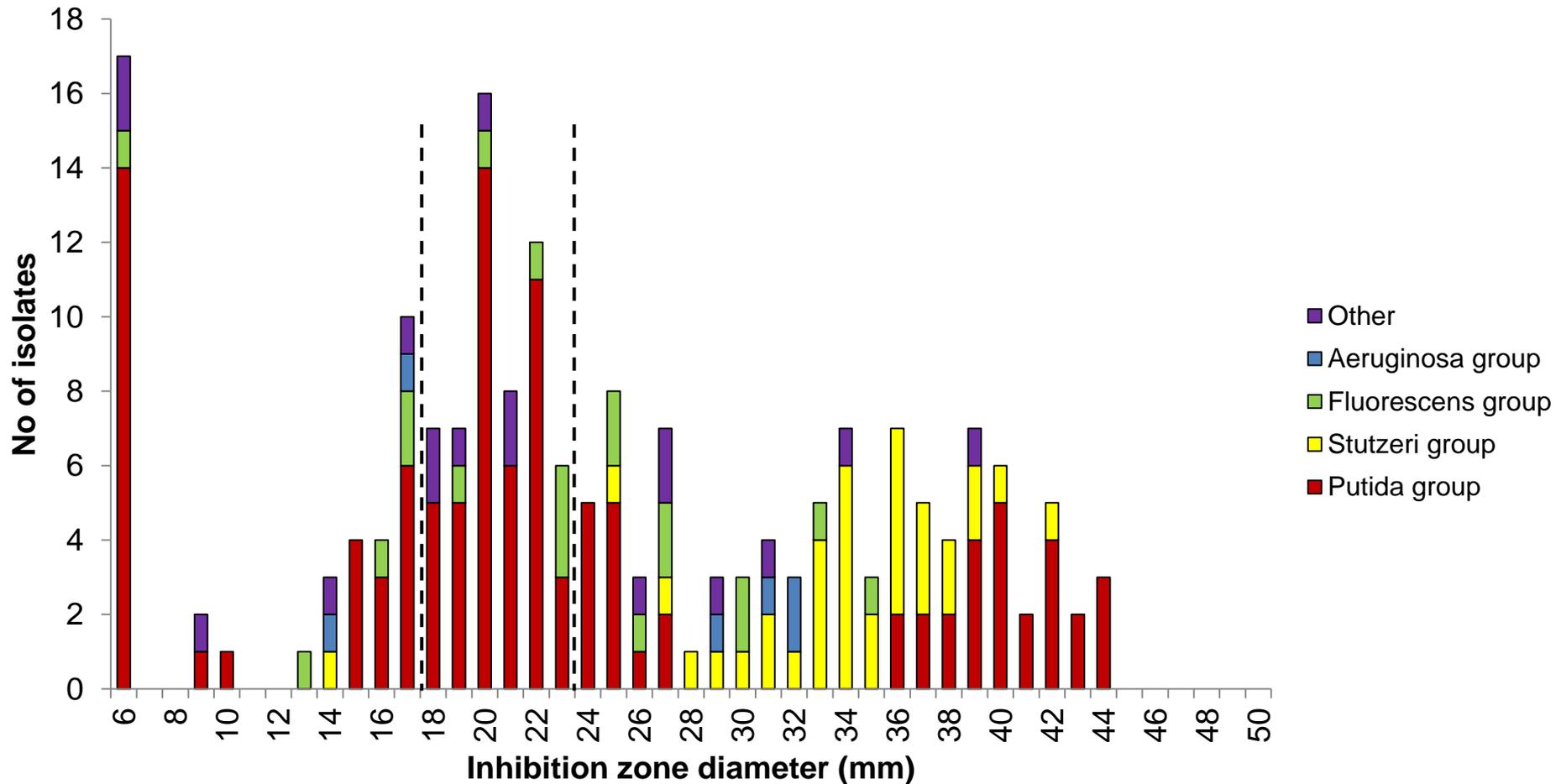
## 189 clinical isolates, *Pseudomonas non-aeruginosa*



| Brytpunkter |                   | ECOFF       |
|-------------|-------------------|-------------|
| MIC         | S ≤ 8, R > 8 mg/L | WT ≤ 8 mg/L |
| Zondiameter | S ≥ 16, R < 16 mm |             |

# Meropenem 10 µg vs. species

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



### Brytpunkter

MIC

$S \leq 2$ ,  $R > 8$  mg/L

Zondiameter

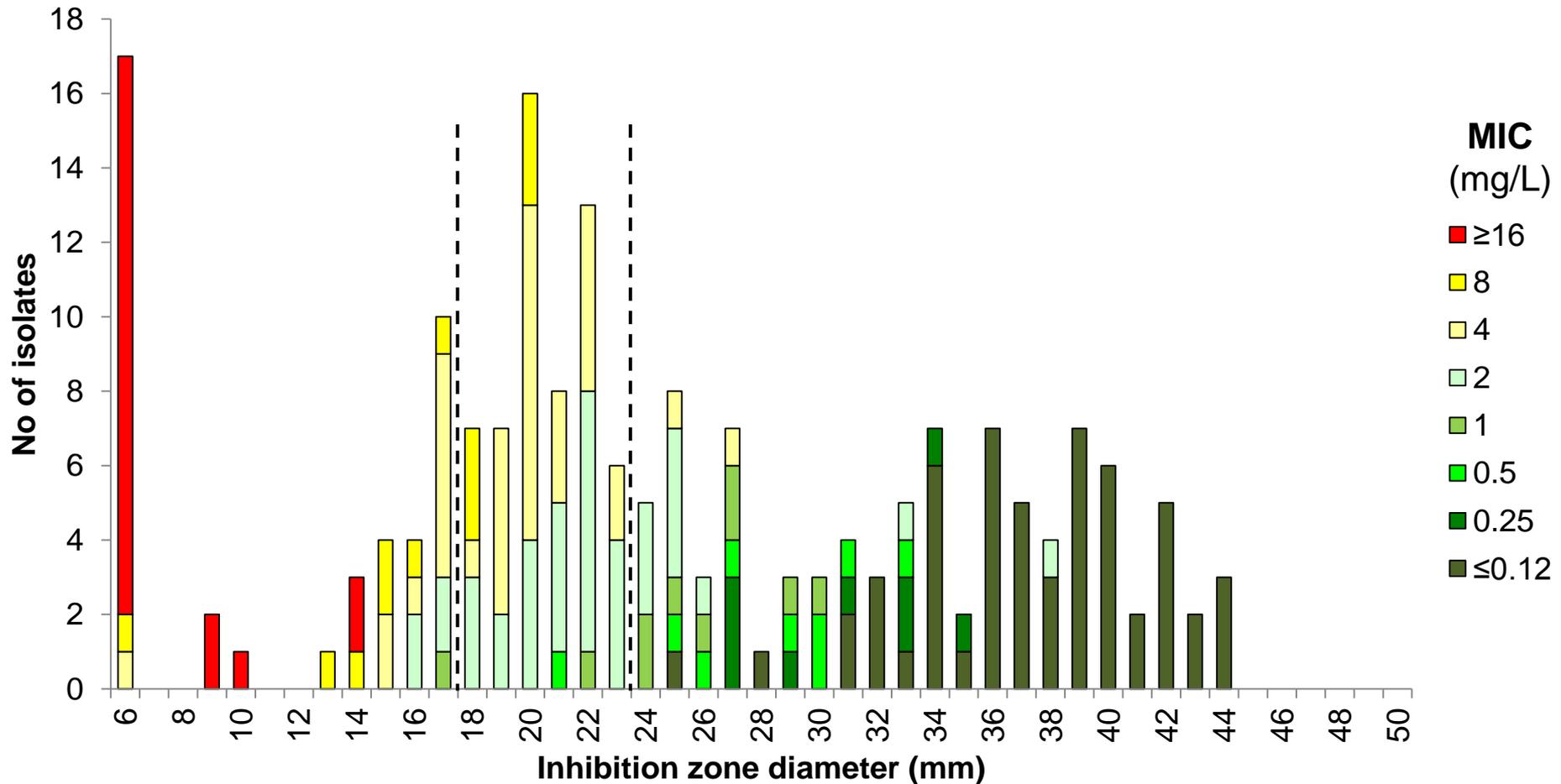
$S \geq 24$ ,  $R < 18$  mm

### ECOFF

$WT \leq 2$  mg/L

# Meropenem 10 µg vs. MIC

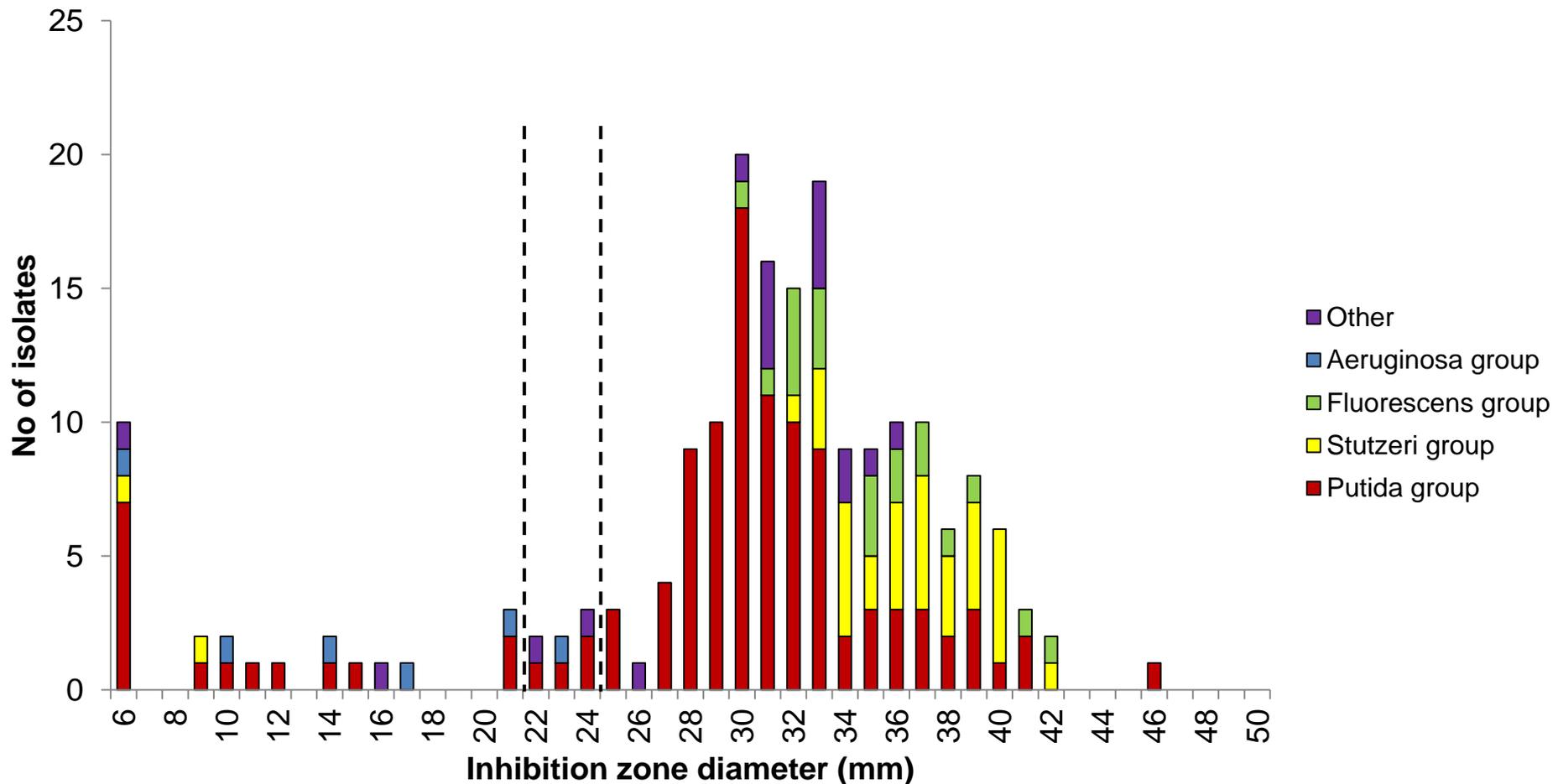
## 191 clinical isolates, *Pseudomonas non-aeruginosa*



|                    |                   |              |
|--------------------|-------------------|--------------|
| <b>Brytpunkter</b> |                   | <b>ECOFF</b> |
| MIC                | S ≤ 2, R > 8 mg/L | WT ≤ 2 mg/L  |
| Zondiameter        | S ≥ 24, R < 18 mm |              |

# Ciprofloxacin 5 µg vs. species

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



### Brytpunkter

MIC S ≤ 0.5, R > 1 mg/L

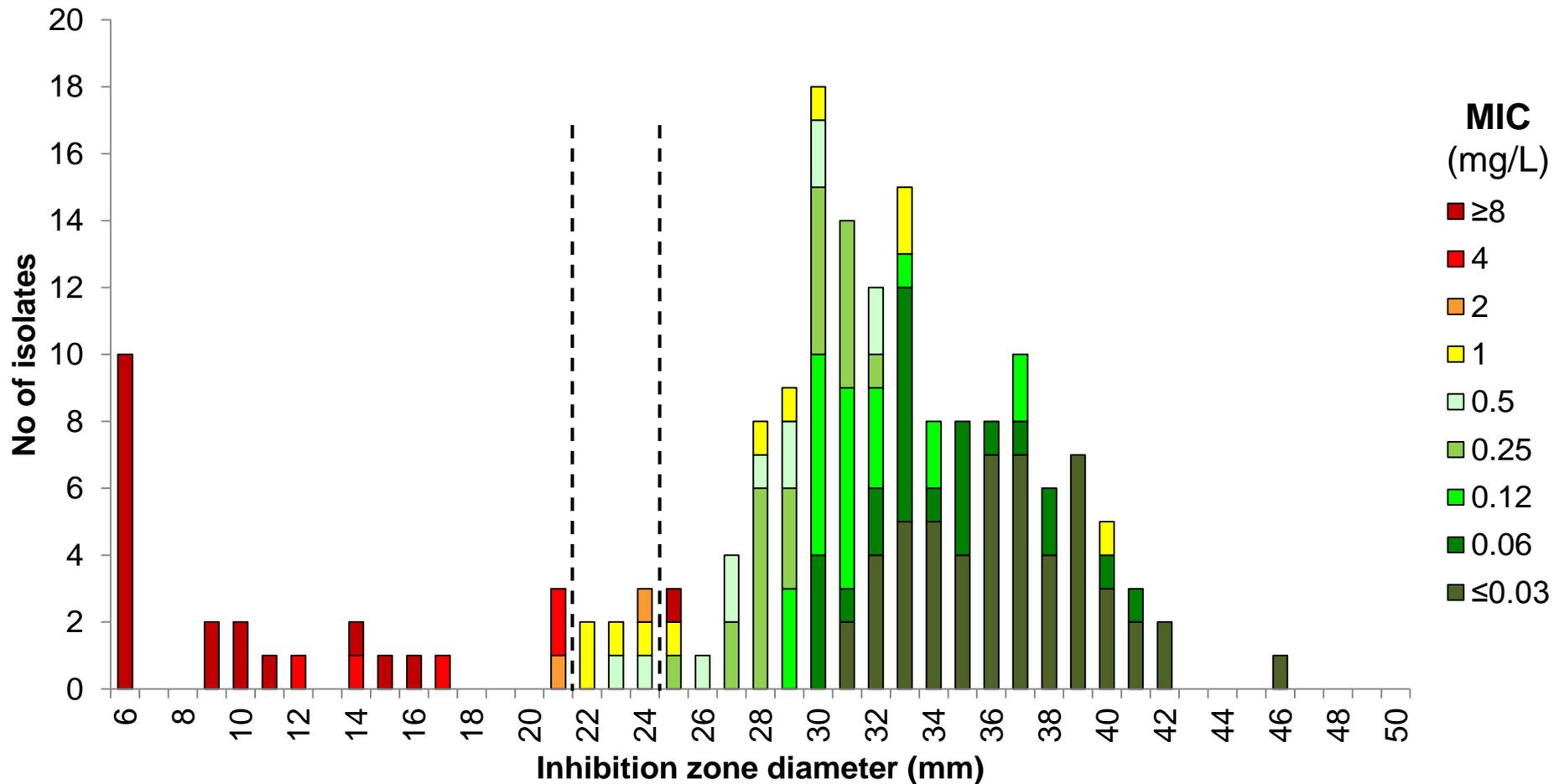
Zondiameter S ≥ 25, R < 22 mm

### ECOFF

WT ≤ 0.5 mg/L

# Ciprofloxacin 5 µg vs. MIC

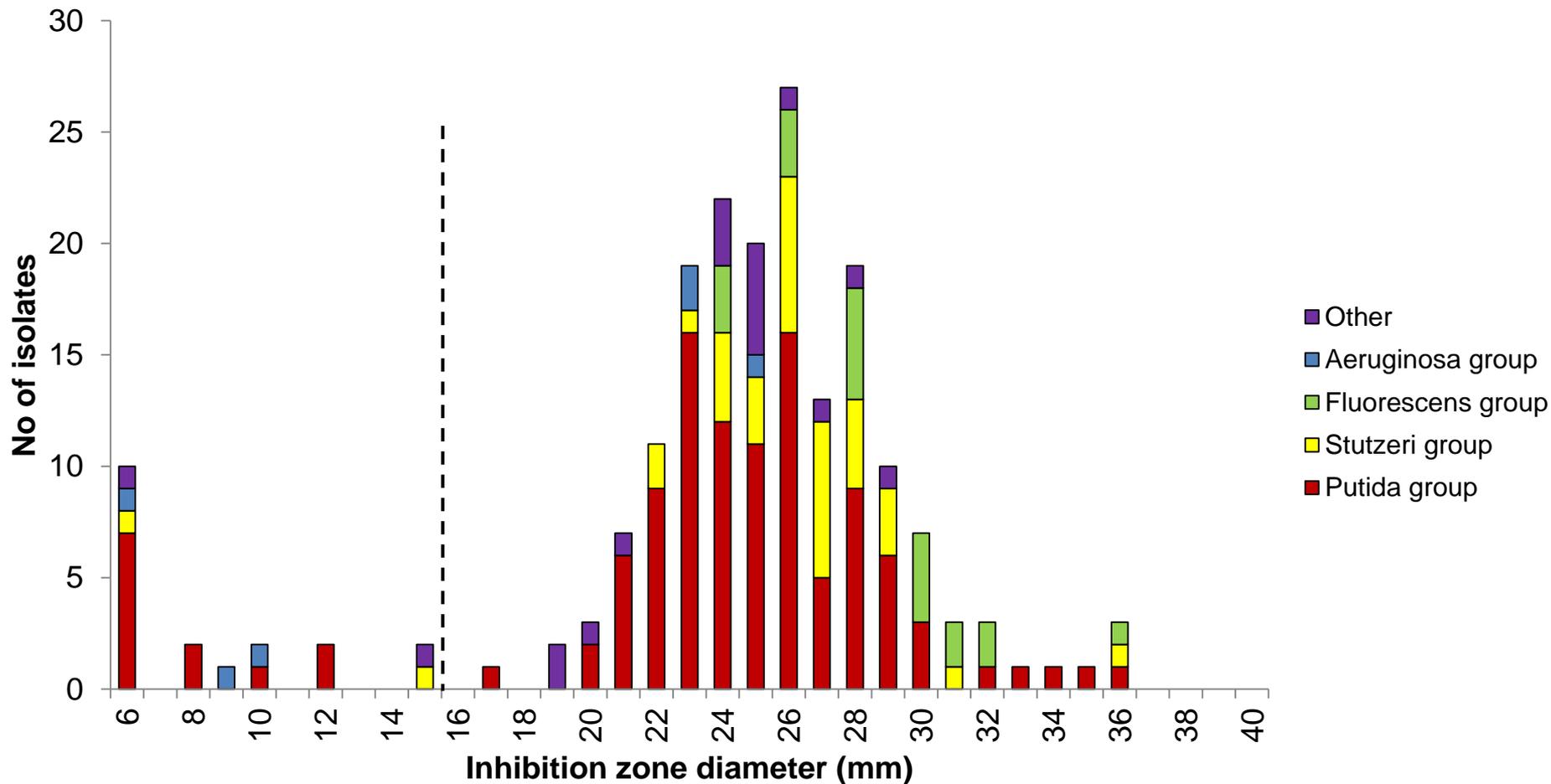
## 173 clinical isolates, *Pseudomonas non-aeruginosa*



|                    |                     |               |
|--------------------|---------------------|---------------|
| <b>Brytpunkter</b> |                     | <b>ECOFF</b>  |
| MIC                | S ≤ 0.5, R > 1 mg/L | WT ≤ 0.5 mg/L |
| Zondiameter        | S ≥ 25, R < 22 mm   |               |

# Tobramycin 10 µg vs. species

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



### Brytpunkter

MIC

$S \leq 4$ ,  $R > 4$  mg/L

Zondiameter

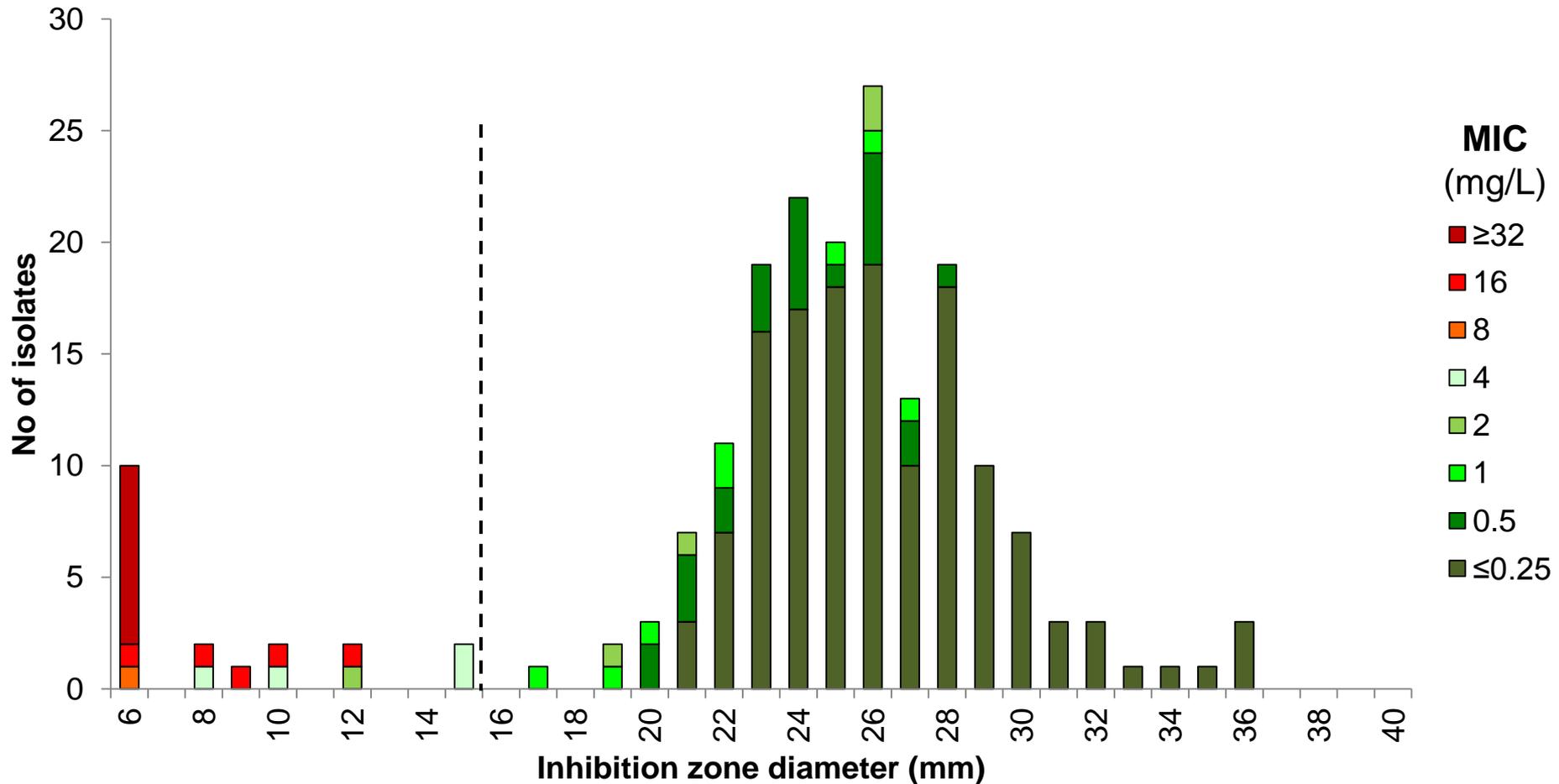
$S \geq 16$ ,  $R < 16$  mm

### ECOFF

$WT \leq 2$  mg/L

# Tobramycin 10 µg vs. MIC

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



|                    |                           |                  |
|--------------------|---------------------------|------------------|
| <b>Brytpunkter</b> |                           | <b>ECOFF</b>     |
| MIC                | S $\leq 4$ , R $> 4$ mg/L | WT $\leq 2$ mg/L |
| Zondiameter        | S $\geq 16$ , R $< 16$ mm |                  |

Lappdiffusion anaerober

# Lappdiffusion anaerober

- Lappdiffusion för *C. difficile* and *B. fragilis*
  - Samarbete med Ulrik Justesen (Danmark) och Elisabeth Nagy (Ungern)
  - *B. fragilis* ATCC 25285 och *C. difficile* ATCC 70057.
  - Variation
    - Media
    - Anaerob miljö (Anaerob-box, Anoxomat vs. behållare med gaskuvert)
    - Temperatur (35°C vs. 37°C)
    - Mellan laboratorier

# Media lappdiffusion anaerober

- Brucella-agar (BBA)
  - Egentillverkade med fårblod (CLSI)
  - Egentillverkade med hästblod
  - BD
  - Oxoid
  - BioMérieux
  - Liofilchem

# Betalaktamas inhibitor- kombinationer

# Betalaktamas inhibitor-kombinationer

- Amoxicillin-klavulansyra
- Ampicillin-sulbaktam
- EUCAST rekommenderar testning med fast koncentration av inhibitor
- Testprodukter innehåller ofta ett fast förhållande (ratio) mellan penicillin och inhibitor.
- Fast koncentration liknar mer de serum-koncentrationer som uppstår vid behandling.

# Betalaktamas inhibitor-kombinationer

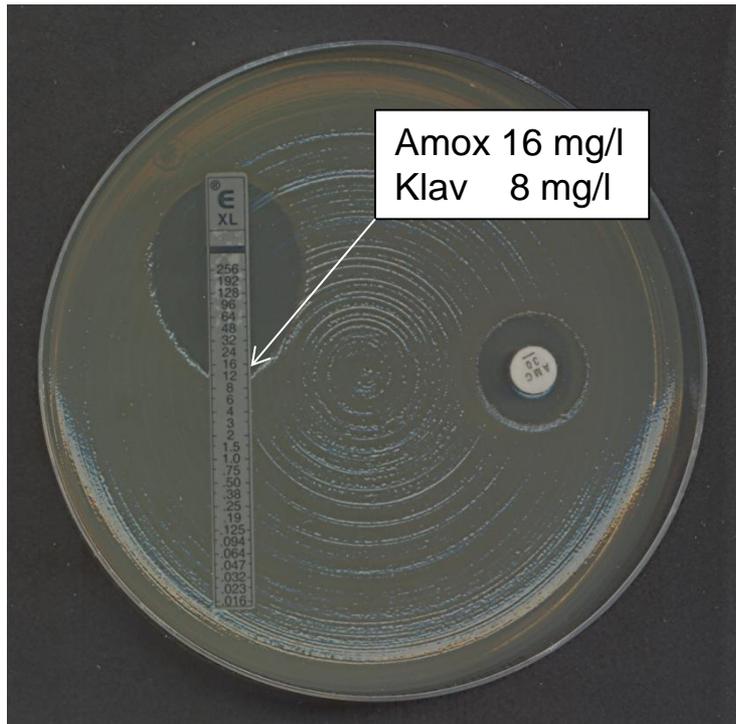
- Utvärdering av QC-gränser
  - *E. coli* ATCC 25922
  - *H. influenzae* NCTC 8468
- MIC-bestämning med buljongspädning
  - Amoxicillin-klavulansyra
    - Fast koncentration 2 mg/l
    - Fast 2:1 ratio
  - Ampicillin-sulbaktam
    - Fast koncentration 4 mg/l
    - Fast 2:1 ratio

# Betalaktamas inhibitor-kombinationer

- Zon/MIC-korrelerat för kliniska isolat
  - Enterobacteriaceae
  - *H. influenzae*
- Buljongspädning med fast ratio och fast koncentration
  - Amoxicillin-klavulansyra
  - Ampicillin-sulbaktam
- Gradienttester för amoxicillin-klavulansyra med fast 2:1 ratio och fast koncentration 2 mg/l.

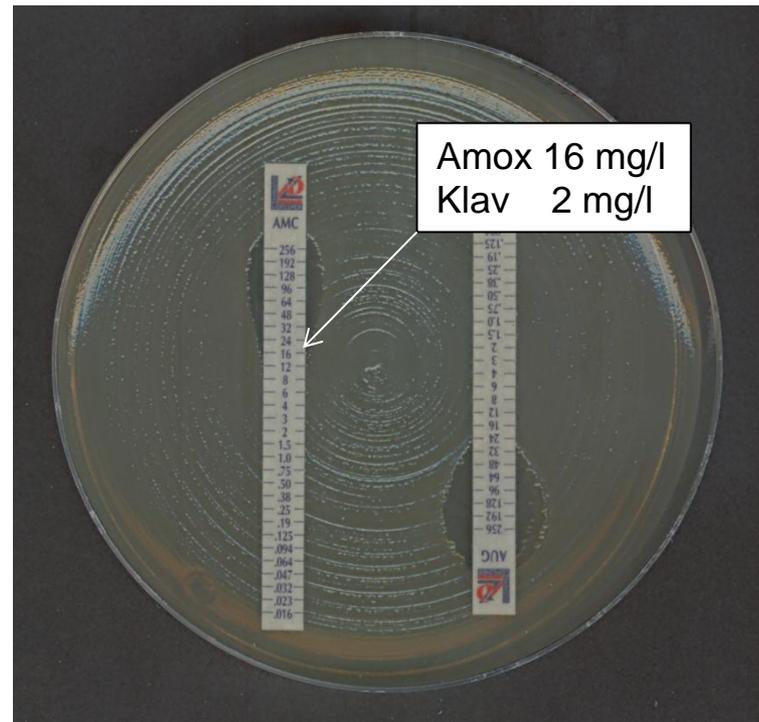
# Amoxicillin-klavulansyra

Fast ratio 2:1



*E. coli* ESBL

Fast konc. 2 mg/l



*E. coli* ESBL

MIC-gränser för *H. influenzae*  
NCTC 8468

# MIC-gränser för NCTC 8468

- Ta fram MIC QC-gränser för *H. influenzae* NCTC 8468
  - Alla antibiotika i QC tabellen.
  - Buljongspädning med MH-F buljong på två laboratorier.
  - Jämförelse med data från JMI med MH-F buljong.
  - Jämförelse med Etest MIC på MH-F agar.