



# News and on-going work EUCAST Development Laboratory (EDL)

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NordicAST workshop 2017

# Breakpoint tables

- Breakpoint table  
(v 7.0, January 2017 and v 7.1, March 2017)
  - New organisms
    - *Aerococcus urinae* and *sanguinicola*
    - *Kingella kingae*
  - New breakpoints
    - Ceftazidime-avibactam
    - Nitroxoline
    - Fosfomycin (zone diameter breakpoints for *E. coli*)
  - Revised breakpoints
    - Fluoroquinolone MIC and zone diameter breakpoints
  - Changed recommendations for screen for methicillin resistance in coagulase-negative staphylococci and *S. pseudintermedius*

# Fosfomycin zone diameter breakpoints

## Enterobacteriaceae

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Fosfomycin iv	32 <sup>2</sup>	32 <sup>2</sup>	200 <sup>B</sup>	24 <sup>C,D</sup>	24 <sup>C,D</sup>
Fosfomycin oral (uncomplicated UTI only)	32 <sup>2</sup>	32 <sup>2</sup>	200 <sup>B</sup>	24 <sup>C,D</sup>	24 <sup>C,D</sup>

2. Agar dilution is the reference method for fosfomycin. MICs must be determined in the presence of glucose-6-phosphate (25 mg/L in the medium). Follow the manufacturers' instructions for commercial systems.

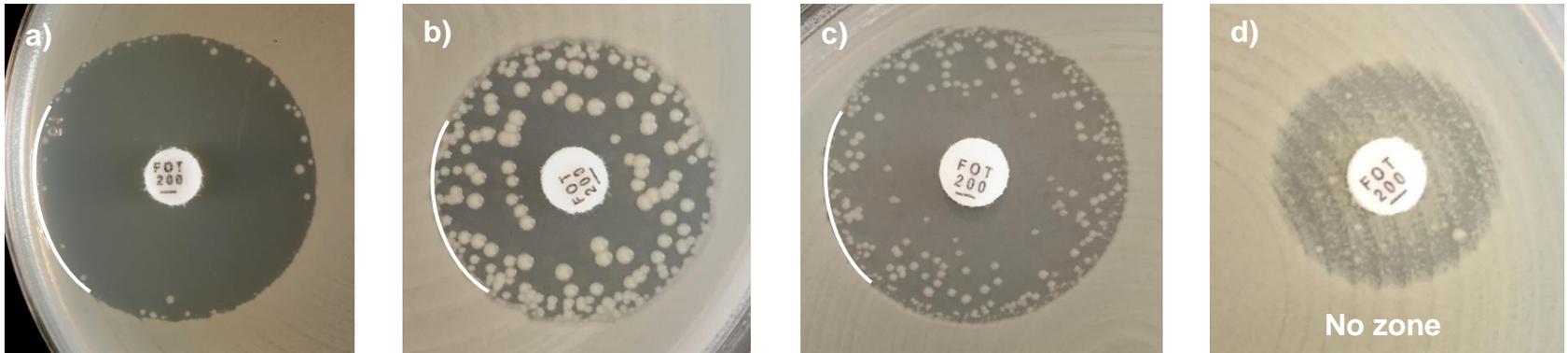
**B.** Fosfomycin 200 µg disks must contain 50 µg glucose-6-phosphate.

**C.** Zone diameter breakpoints apply to *E. coli* only. For other Enterobacteriaceae, use an MIC method.

**D.** Ignore isolated colonies within the inhibition zone.

# Reading of fosfomycin zones

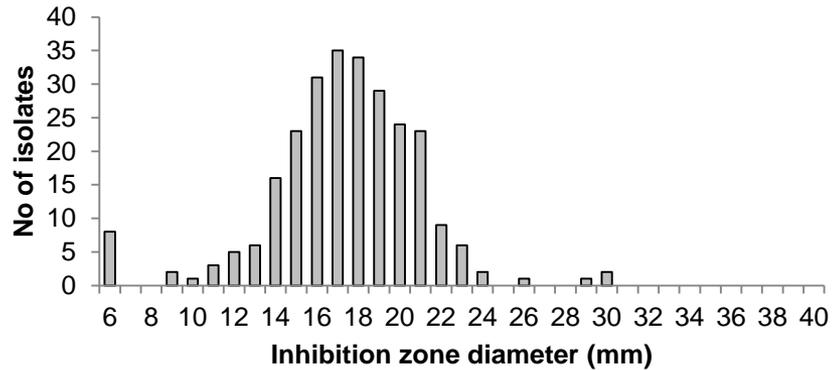
Ignore isolated colonies within the inhibition zone and read the outer zone edge.



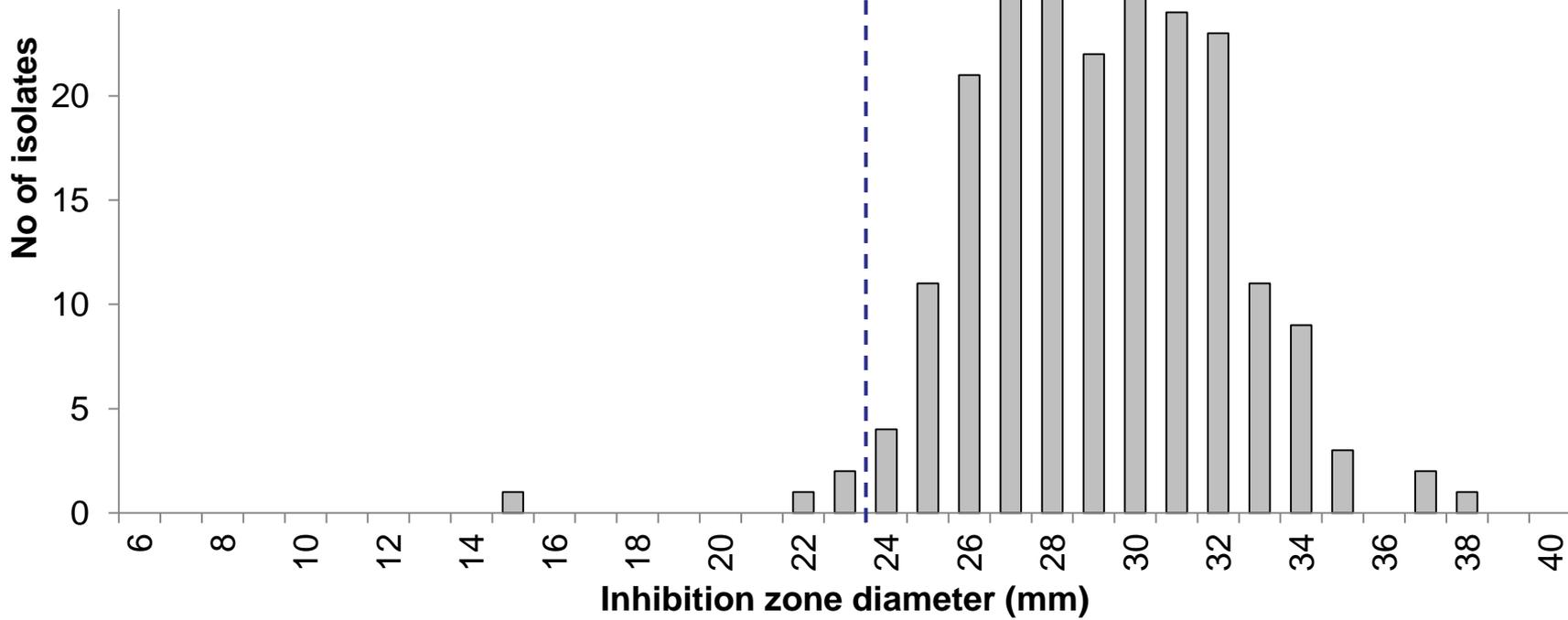
a-c) Ignore all colonies and read the outer zone edge  
d) Record as no inhibition zone



**Fosfomicin 200 µg**  
***K. pneumoniae***



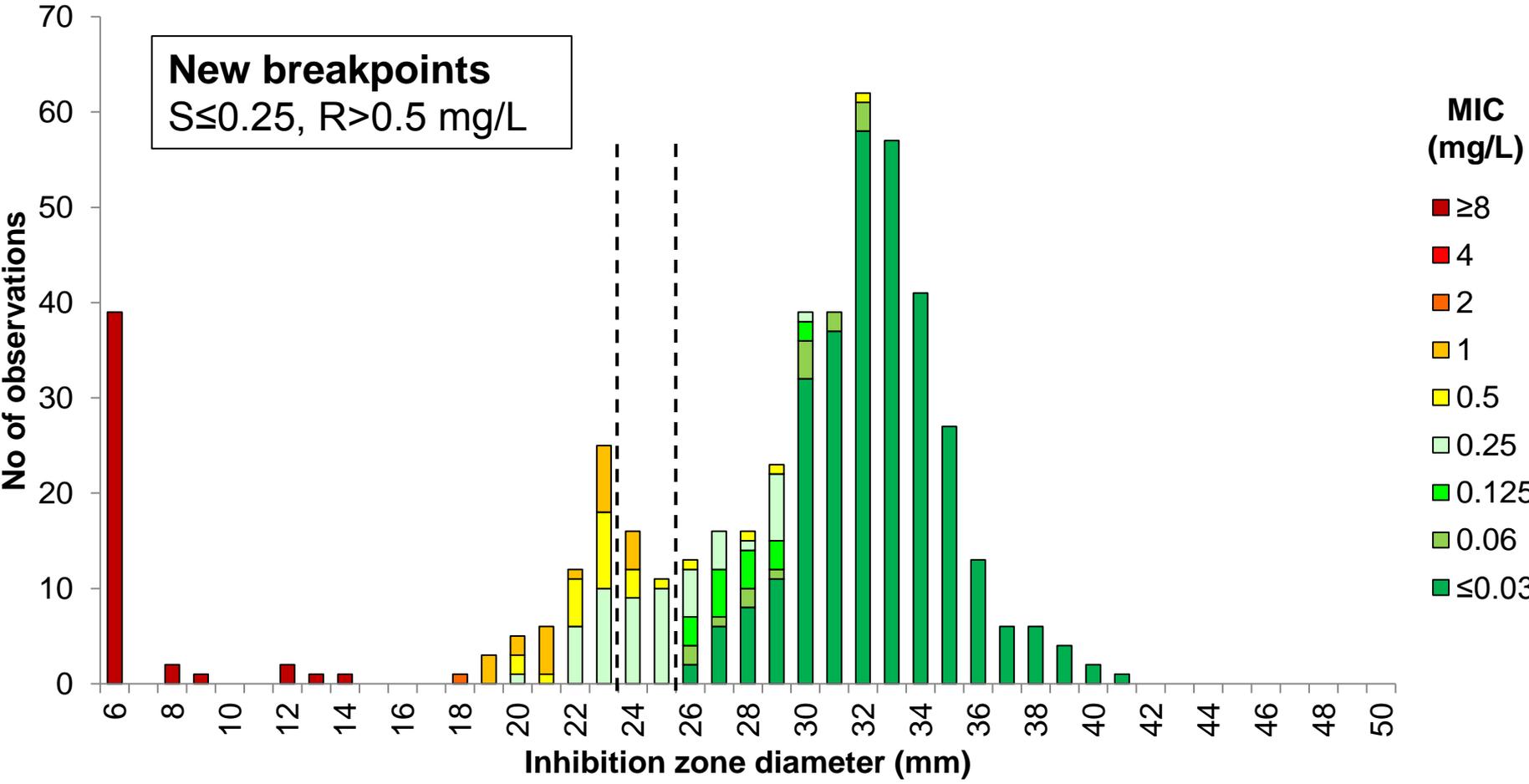
**Clindamycin 200 µg**  
**consecutive isolates**





Revised fluoroquinolone breakpoints

Ciprofloxacin 5 µg vs. MIC  
Enterobacteriaceae, 471 isolates (490 correlates)









# Screen for methicillin resistance in staphylococci

## EUCAST breakpoint table v 7.1

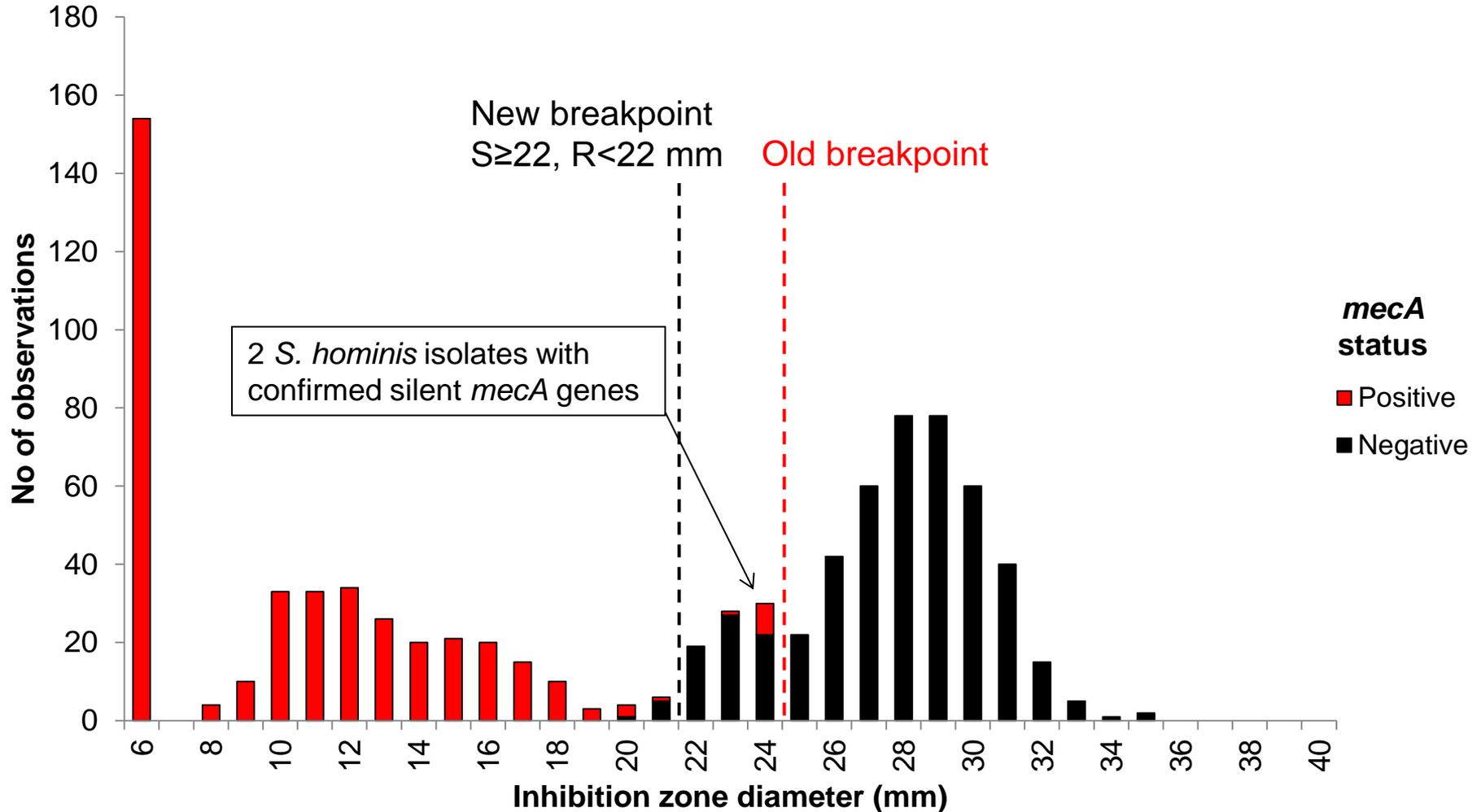
Cefoxitin (screen), <i>S. aureus</i> and coagulase-negative staphylococci other than <i>S. epidermidis</i>	Note <sup>3,4</sup>	Note <sup>3,4</sup>	30	22 <sup>A,B</sup>	22 <sup>A,B</sup>
Cefoxitin (screen), <i>S. epidermidis</i>	Note <sup>4</sup>	Note <sup>4</sup>	30	25 <sup>A,B</sup>	25 <sup>A,B</sup>
Cefoxitin (screen), <i>S. pseudintermedius</i>	NA	NA	30	Note <sup>E</sup>	Note <sup>E</sup>

**B.** If coagulase-negative staphylococci are not identified to species level use zone diameter breakpoints  $S \geq 25$ ,  $R < 25$  mm.

**E.** Cefoxitin screen for methicillin resistance in *S. pseudintermedius* is less predictive of the presence of *mecA* than in other staphylococci. Use the oxacillin 1  $\mu$ g disk with zone diameter breakpoints  $S \geq 20$ ,  $R < 20$  mm to screen for methicillin resistance.

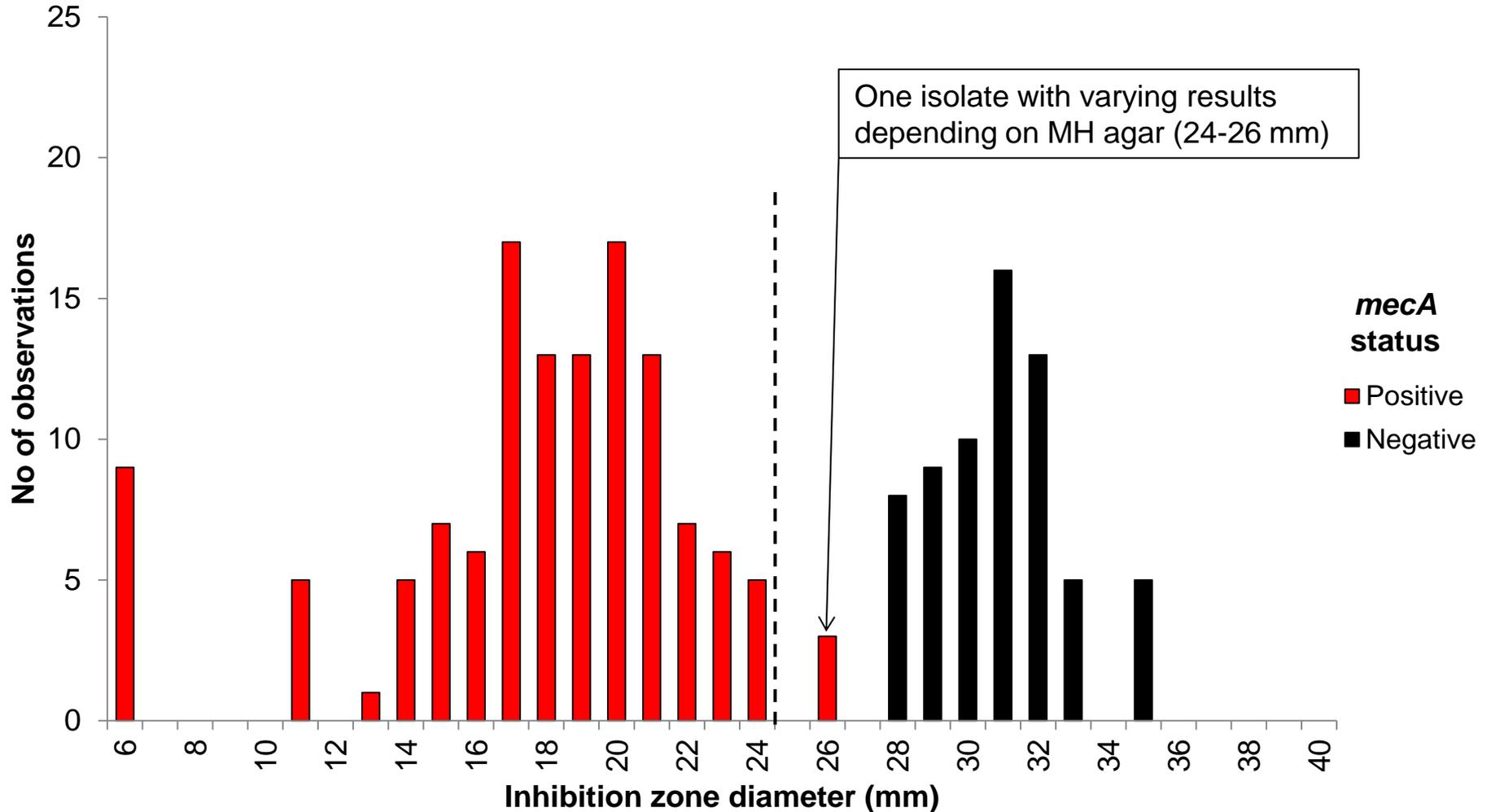
# Coagulase negative staphylococci other than *S. epidermidis*

Cefoxitin 30 µg vs. *mecA* status



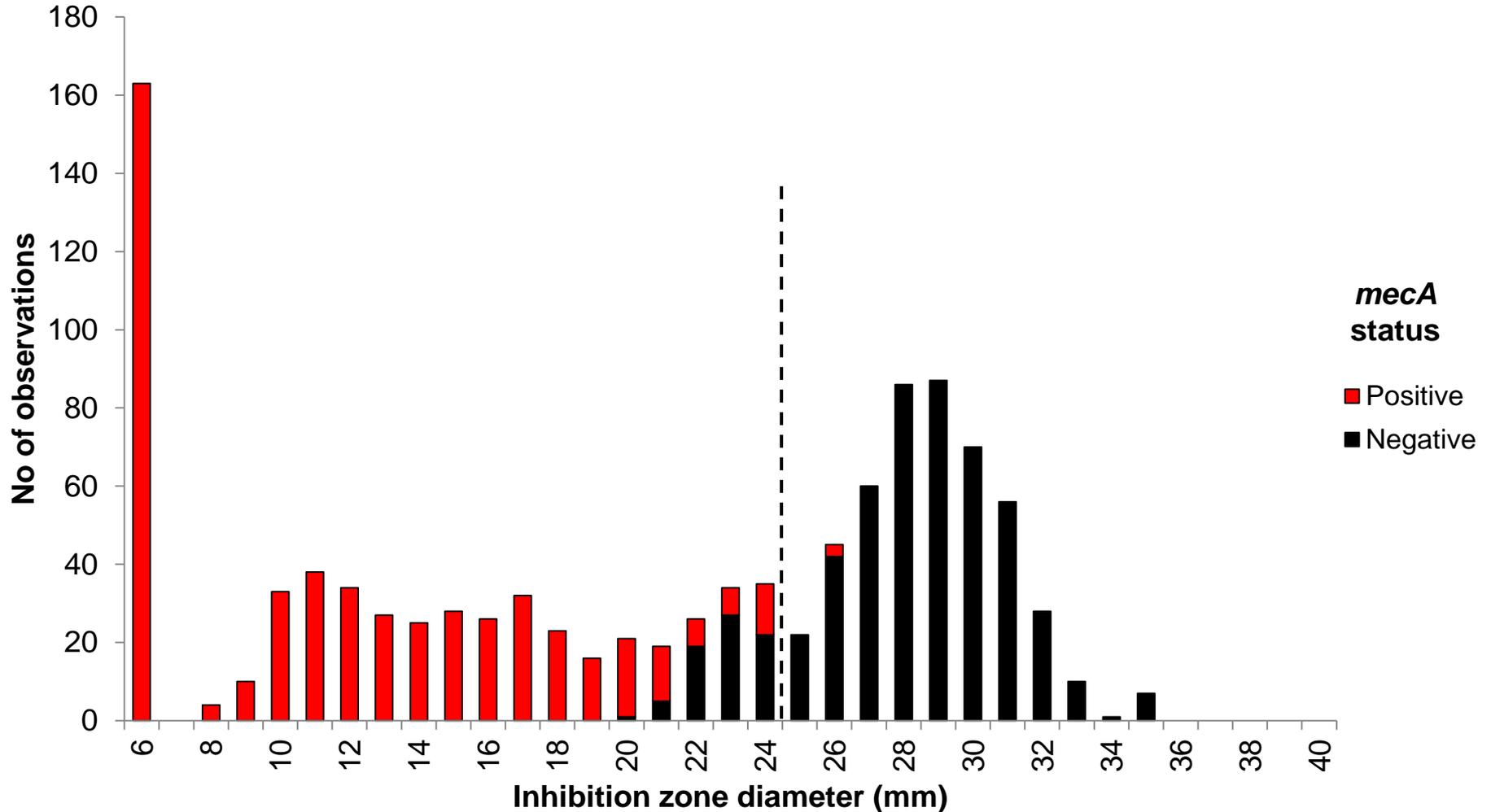
# *S. epidermidis*

## Cefoxitin 30 $\mu$ g vs. *mecA* status



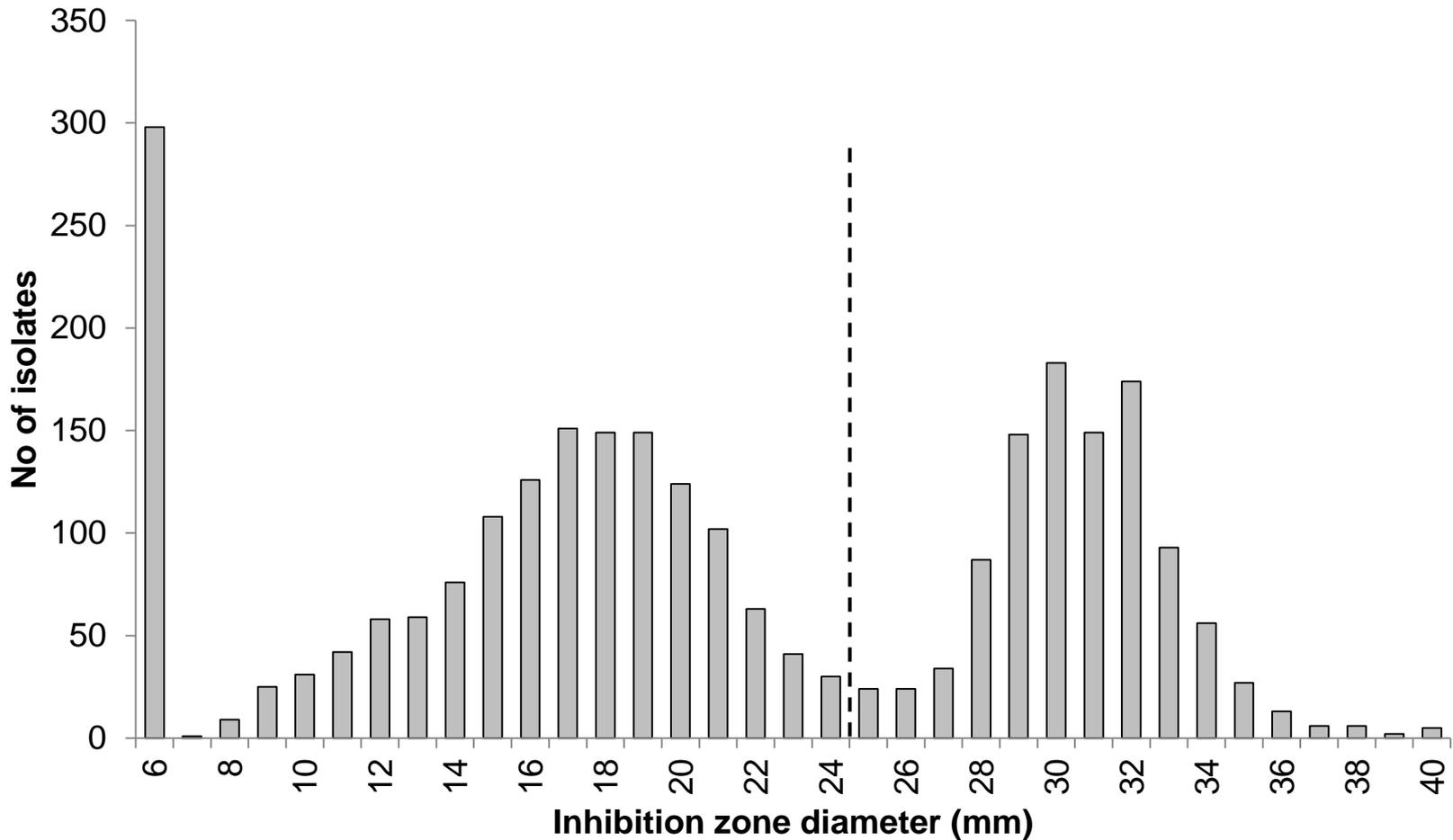
# All coagulase negative staphylococci

Cefoxitin 30 µg vs. *mecA* status



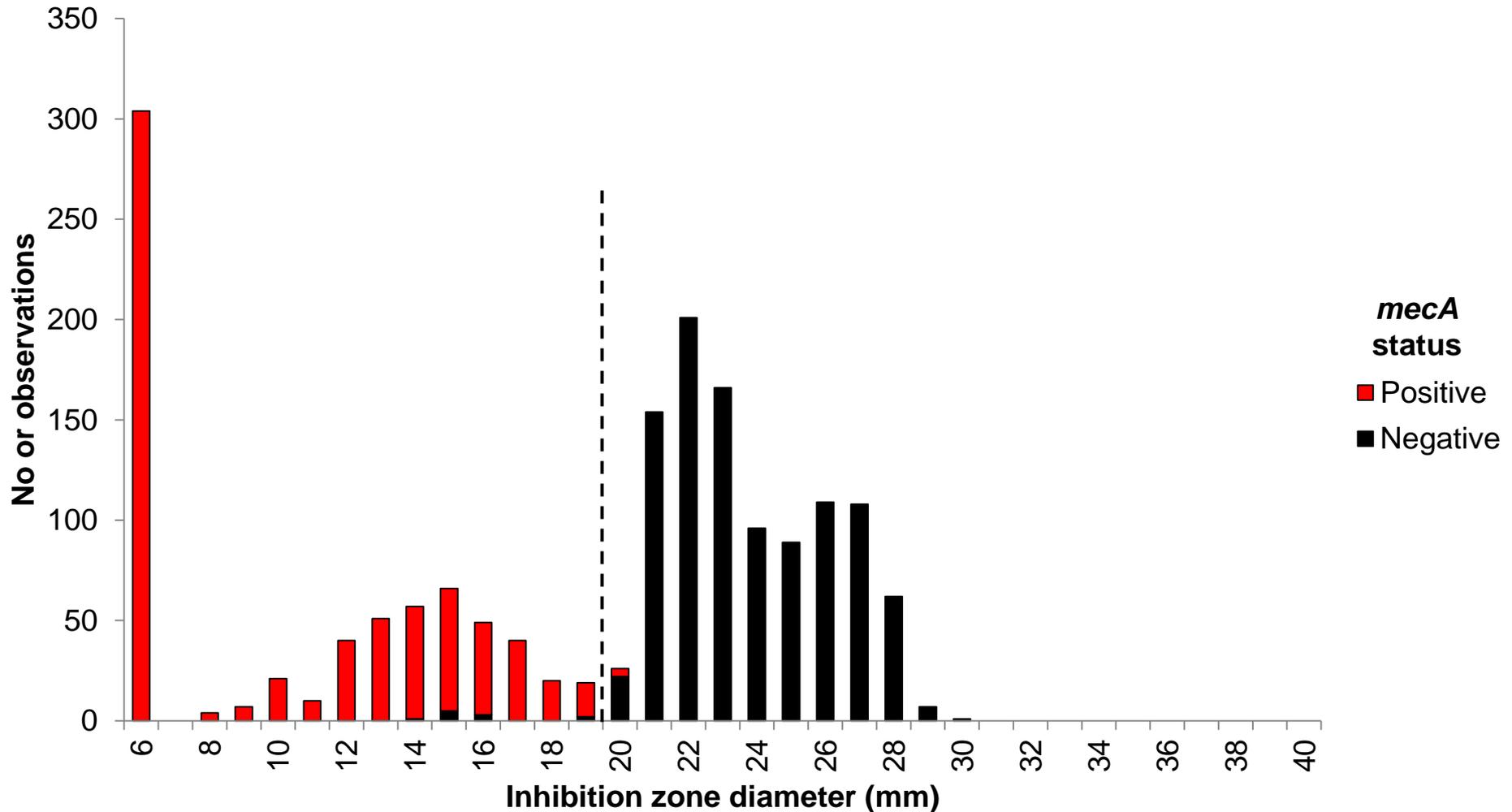
# *S. epidermidis* consecutive isolates

Cefoxitin 30 µg



# *S. pseudintermedius* - Oxacillin screen

Oxacillin 1  $\mu$ g vs. *mecA* status



# QC tables

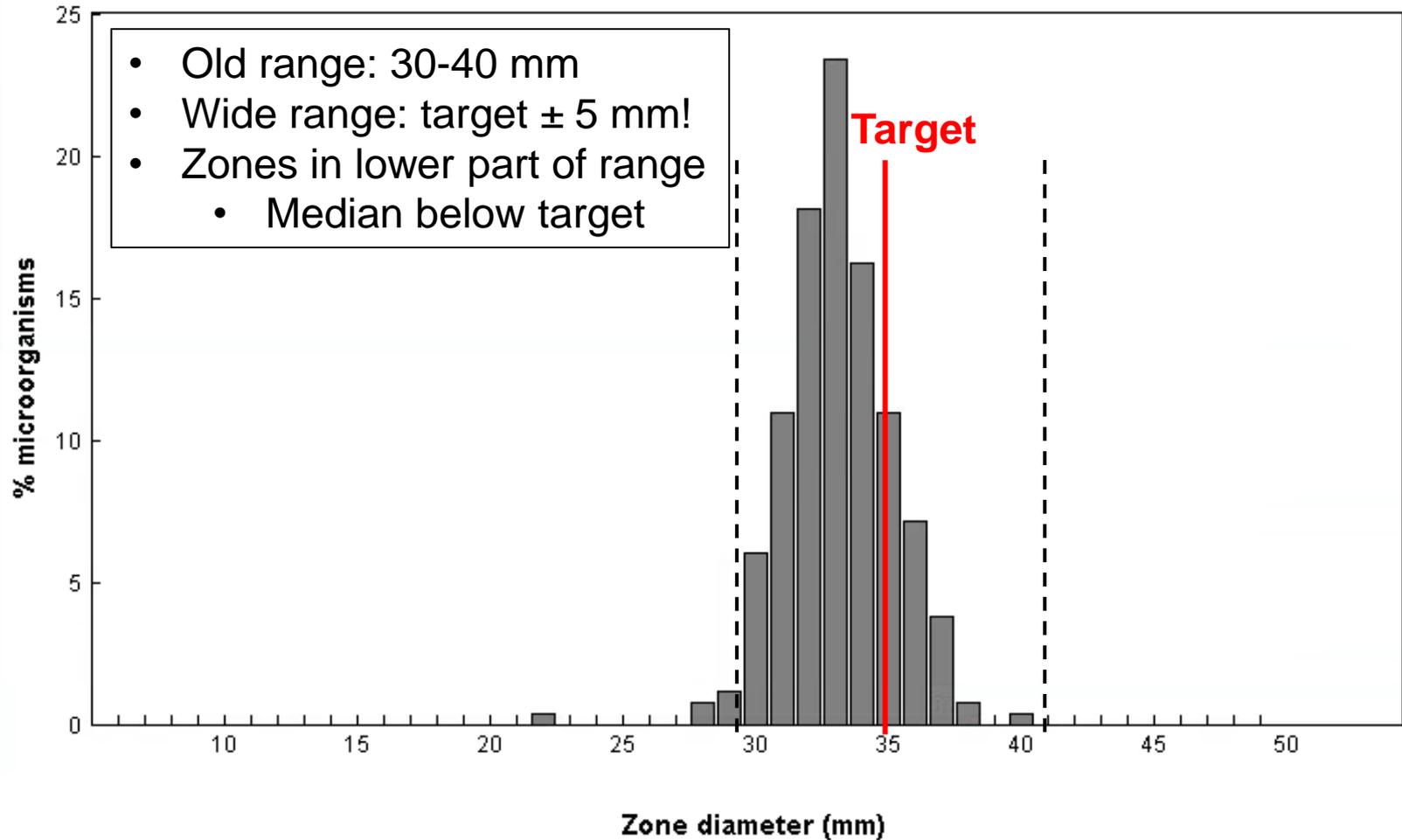
- QC tables (v 7.0, January 2017)
  - New agents added
    - Ceftazidime-avibactam
    - Nitroxoline
  - New criteria
    - Resistant QC strain for colistin (*E. coli* NCTC 13846)
    - Range and target for *K. pneumoniae* ATCC 700603 and piperacillin-tazobactam 30-6 µg
  - Several zone diameter ranges evaluated and some revised
    - *E. coli* ATCC 25922 with cefixime 5 µg and ciprofloxacin 5 µg

## Ciprofloxacin / Escherichia coli ATCC 25922

International wild type zone diameter distribution - Reference database 2017-05-18

### 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

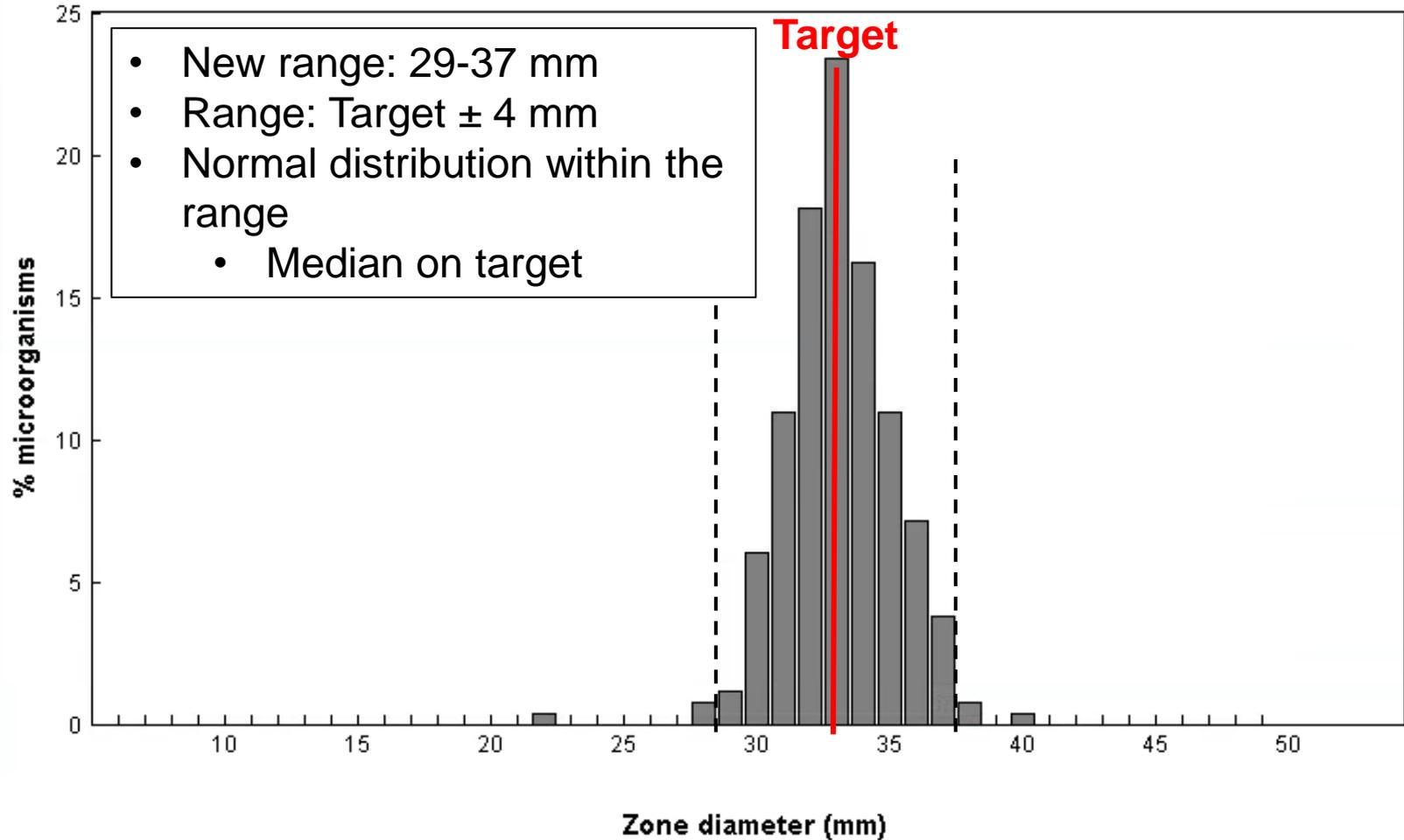


## Ciprofloxacin / Escherichia coli ATCC 25922

International wild type zone diameter distribution - Reference database 2017-05-18

### 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



# Updates on website

- All “Calibration and validation files” on website updated.
- Warnings concerning antimicrobial susceptibility testing products or procedures.
  - Evaluation of disks from 9 manufacturers – poor performance detected
  - Problems with piperacillin-tazobactam gradient tests
  - Problems with colistin gradient tests



# EUCAST

EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases



## AST of bacteria

[Organization](#)

[EUCAST News](#)

[Clinical breakpoints](#)

[Expert rules and intrinsic resistance](#)

[Resistance mechanisms](#)

[Guidance documents](#)

[Consultations](#)

[MIC distributions and ECOFFs](#)

[Zone distributions and ECOFFs](#)

### AST of bacteria

[Media preparation](#)

[MIC determination](#)

[Disk diffusion methodology](#)

[Disk diffusion implementation](#)

[Compliance of manufacturers](#)

[Breakpoint tables](#)

[QC Tables](#)

[Calibration and validation](#)

[Warnings!](#)

[Guidance documents](#)

[Projects and data submission](#)

[MIC testing services from EUCAST](#)

[Previous versions of documents](#)

[AST of mycobacteria](#)

[AST of fungi](#)

[AST of veterinary pathogens](#)



AST of bacteria



## Antimicrobial susceptibility testing

Antimicrobial susceptibility testing is performed with phenotypic or genotypic methods. The basis of phenotypic methods is the minimum inhibitory concentration (MIC). Clinical MIC breakpoints determine whether the organism is categorised as susceptible, intermediate or resistant to the agent in question. Other methods should be calibrated to reference MIC methods.

Users of EUCAST breakpoints should use the [EUCAST disk diffusion method](#) or other susceptibility testing systems calibrated to EUCAST breakpoints and terminology in accordance with EUCAST breakpoint tables.

For videos on how to perform disk diffusion testing according to EUCAST - [CLICK here!](#)

For more information - [CLICK here.](#)

- **Media preparation**  
On how to prepare media for MIC and disk testing
- **MIC determination** of nonfastidious and fastidious organisms  
Broth microdilution methodology according to ISO and EUCAST
- **Disk diffusion methodology**  
Detailed description of the EUCAST disk diffusion test
- **Disk diffusion implementation**  
Guidance documents on how to implement the disk diffusion test
- **Compliance of manufacturers**  
Compliance of manufacturers of susceptibility testing products with EUCAST guidelines
- **Breakpoint tables**  
Current MIC and zone diameter breakpoint tables
- **QC tables**  
Current tables of MIC and zone diameter ranges for quality control strains
- **Calibration and validation**  
Data used in the development and calibration of EUCAST disk diffusion breakpoints

# On-going work (I)

- New agents
- Zone diameter breakpoints for “old agents”
  - Temocillin
  - Fosfomicin (Enterobacteriaceae other than *E. coli*)
- Colistin MIC testing
  - Agar dilution
  - Additional BMD methods?
- New evaluation of disks from 9 manufacturers

# Variability in disk quality

1 = 1st study, 2 = Follow-up study

Antimicrobial disk	Bio-Rad		Liofilchem		BD		Abtek		SirScan		Oxoid		HiMedia		Bioanalyse		Mast	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Benzylpenicillin 1 unit					L				H	H			NA	NA	H	H		
Amoxicillin-clav. 30 µg	H	H*					L						H	H		L		
Piperacillin-tazo. 36 µg							L	L	H				NA	NA				
Oxacillin 1 µg			L		L				L				H	H	L			
Mecillinam 10 µg							L		H				H		H			
Cefotaxime 5 µg							NA		L				NA	NA				
Cefoxitin 30 µg	H*	H*	H	H*			NA	L					L*	L*		L		
Ceftazidime 10 µg							L	L					L	H				
Meropenem 10 µg	H		H*				L	L			H		H					
Ciprofloxacin 5 µg	L				L		L	L					H	H*		L	L	
Norfloxacin 10 µg							L		L				H*	H				
Pefloxacin 5 µg			L	L	L		NA	NA	NA				H					
Gentamicin 10 µg					H		L		NA				H	H				
Tobramycin 10 µg	NA	NA	H										H*	H*				
Erythromycin 15 µg			L		L		L		L				H	H	L*	L		
Tetracycline 30 µg			L	L*	L*		L		L*					L	L		L	

Mean value within  $\pm 1$  mm of the target value

Mean value  $>1$  mm but within  $\pm 2$  mm of the target value

Mean value  $>2$  mm from target value but still within the QC range

Mean value out of the QC range

Disk included in first study, but not supplied for second study

NA = Not Available

H = High, mean value  $>1$  mm above target

L = Low, mean value  $>1$  mm below target

\* One or more readings out of QC range

# On-going work (II)

- Zone diameter breakpoints for early reading (4-8 h incubation) of disk diffusion AST
  - Additional organisms
  - Different blood culturing systems
  - Multi-laboratory validation
- Evaluation of ESBL confirmation disks with cefotaxime 5 µg and ceftazidime 10 µg ± clavulanic acid
- β-lactam resistance in *H. influenzae*

# $\beta$ -lactam resistance in *H. influenzae*

## 1. Aminopenicillin breakpoints

- MIC breakpoints intersect the low-level resistant population
- Methodological problems with disk diffusion

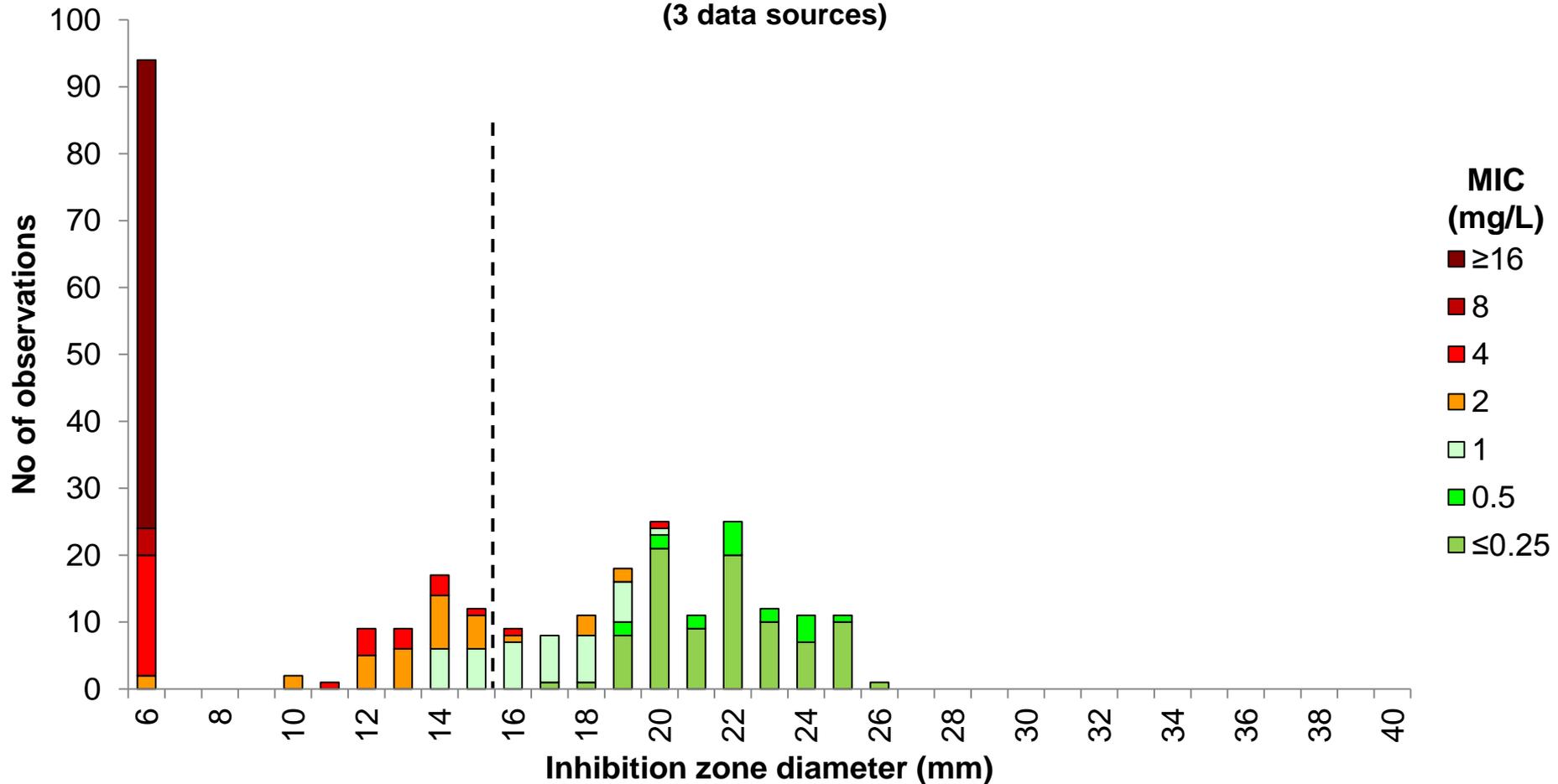
## 1. Improvement of screening algorithm

- Evaluation of benzylpenicillin 1 unit  $\pm$  clavulanic acid to separate isolates with  $\beta$ -lactamase only from those with  $\beta$ -lactamase and altered PBP3

# Ampicillin 2 $\mu$ g vs. MIC

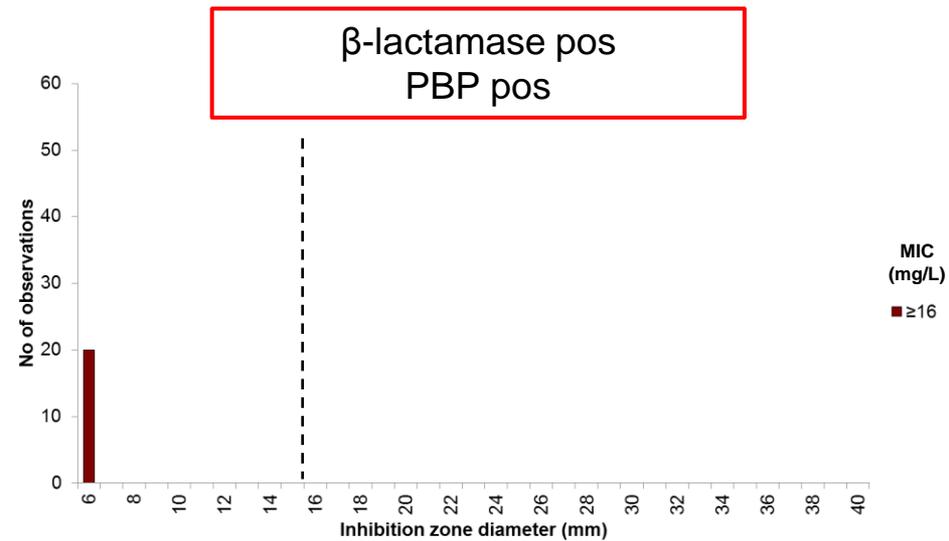
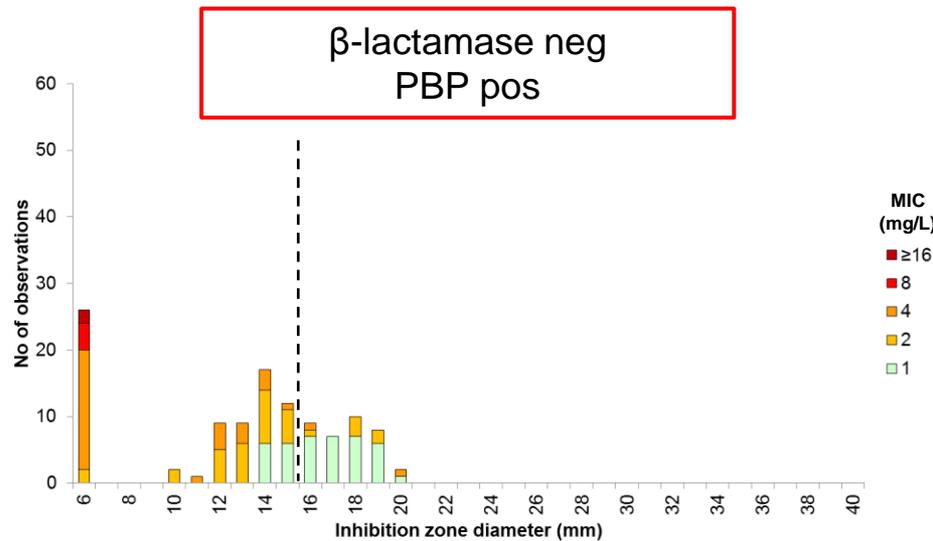
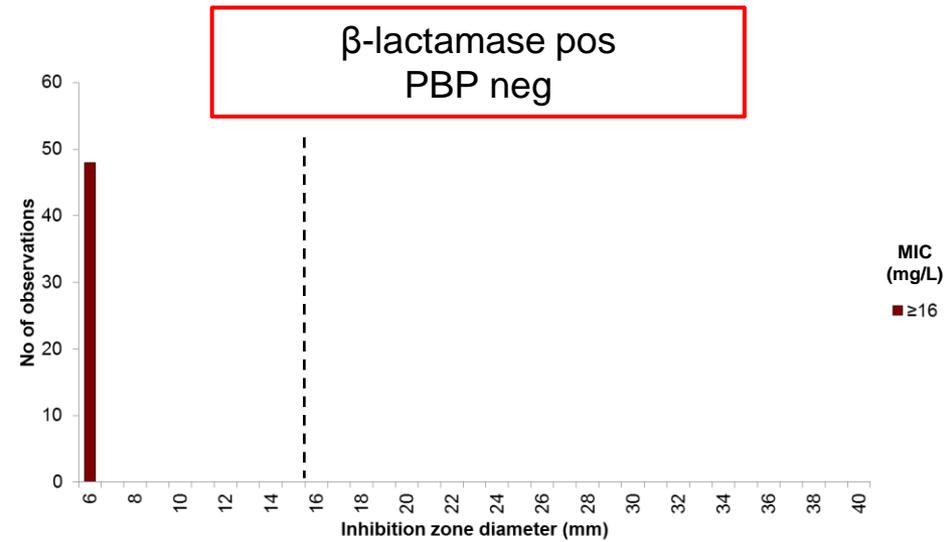
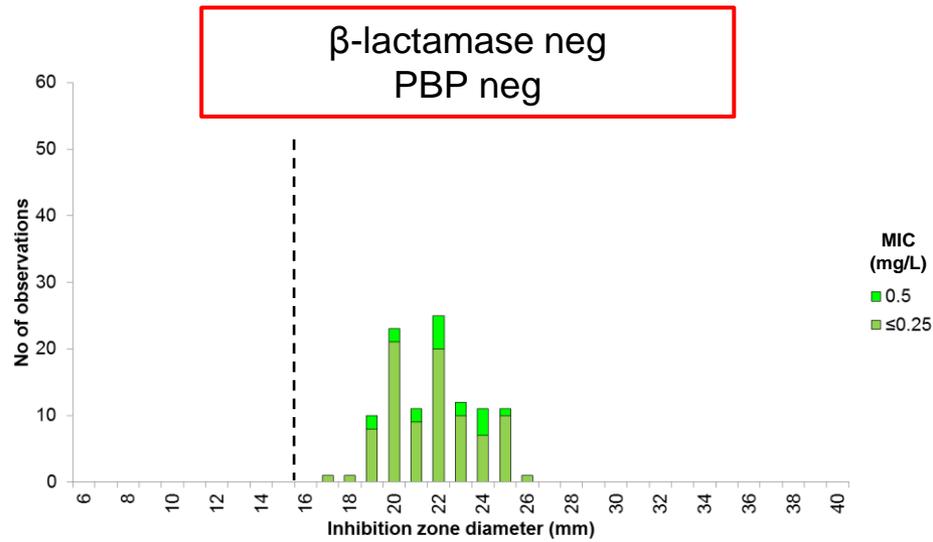
## *H. influenzae*, 143 isolates (286 correlates)

(3 data sources)



Breakpoints		ECOFF
MIC	$S \leq 1$ , $R > 1$ mg/L	1 mg/L
Zone diameter	$S \geq 16$ , $R < 16$ mm	

# Ampicillin vs. resistance mechanism



# On-going work (III)

- Organisms lacking EUCAST breakpoints and methods:
    - *Aeromonas* spp.
    - *Plesiomonas shigelloides* } MIC-zone diameter correlations during 2016-2017
  - *Vibrio* spp.
  - *Nocardia* spp.
  - Fast-growing anaerobes
- } Method evaluation during 2017
- HACEK organisms other than *Kingella kingae*
- *Streptomyces* spp.
- *Leuconostoc* spp.
- *Lactobacillus* spp.
- *Abiotrophia* and *Granulicatella* spp.
- *Gemella* spp.
- *Achromobacter* spp.
- *Bacillus* spp. other than *B. anthracis*
- *Pediococcus* spp.
- } From 2018

# EDL resources

- Educational videos (in collaboration with WHO) on the EUCAST disk diffusion method:
  1. Preparation of inoculum
  2. Inoculation of agar plates for disk diffusion
  3. Application of antibiotic disks and incubation of plates
  4. Reading of inhibition zone diameters
  5. Guidance on the use of the breakpoint table

www.youtube.com



# EUCAST disk diffusion antimicrobial susceptibility testing method

## Preparation of inoculum

0:02 / 2:33

### EUCAST videos (English)

Gunnar Kahlmeter · 1/5 videos



1 Preparation of inoculum (english)

Gunnar Kahlmeter

2



2 Inoculation english

Gunnar Kahlmeter

3



3 Application of disks and incubation english

Gunnar Kahlmeter

4



4 Reading of zones english

Gunnar Kahlmeter

5



5 Guidance on breakpoint table english

Gunnar Kahlmeter

All videos are with English speaker, but subtitles are available in Arabic, Czech, French, German, Portuguese, Russian, Spanish and Turkish.

More translations are to come.

# EDL resources

- New course in disk diffusion methodology
  - Clinical Microbiology Växjö, October 2017
  - To be announced on [www.nordicast.org](http://www.nordicast.org)
- MIC determination with broth microdilution
  - Information on available panels, prices and form for ordering on <http://www.nordicast.org/metoder>
  - Results usually within 3 working days

# Questions and/or problem?

Contact us!

[erika.matuschek@escmid.org](mailto:erika.matuschek@escmid.org)

