



EUCAST

EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

# *Haemophilus influenzae* Breakpoints & methods

Erika Matuschek, PhD

EUCAST Laboratory for AST

# Susceptibility testing media

## Haemophilus Test Medium, **HTM** (CLSI)

- Mueller-Hinton agar/broth
- 5 g/L yeast extract
- 15 mg/L bovine or porcine hematin
- 15 mg/L  $\beta$ -NAD

## Mueller-Hinton F, **MH-F** (EUCAST)

- Mueller-Hinton agar/broth
- 5% defibrinated horse blood
- 20 mg/L  $\beta$ -NAD

# EUCAST MH-F medium

- A common media for fastidious organisms
  - *H. influenzae*
  - *S. pneumoniae*
  - $\alpha$ - and  $\beta$ -haemolytic streptococci
  - Viridans group streptococci
  - *Moraxella catarrhalis*
  - *Listeria monocytogenes*
  - *Pasteurella multocida* (v 3.0)
  - *Campylobacter jejuni* and *coli* (v 3.0)
  - *Corynebacterium* spp. (during 2013)

# Development of MH-F

- Supplementation with blood
  - Good growth of *H. influenzae* with 5% horse blood and 20 mg  $\beta$ -NAD /L
  - Sheep blood required 5x  $\beta$ -NAD concentration
  - The horse blood must be mechanically defibrinated!
- $\beta$ -NAD concentration
  - 20 mg/L chosen to ensure sufficient growth with varying manufacturers
  - Purity of  $\geq 98\%$  recommended

# EUCAST methodology

	Disk diffusion	Broth microdilution
Media	MH-F	MH-F broth*
Inoculum	McFarland 0.5	$5 \times 10^5$ cfu/mL
Incubation	16-20 h $35 \pm 1^\circ\text{C}$ 5% CO <sub>2</sub>	16-20 h $35 \pm 1^\circ\text{C}$ Ambient air (sealed panels)

## \* MH-F broth

Mueller-Hinton broth with 5% lysed horse blood and 20 mg/L  $\beta$ -NAD. The horse blood is lysed by repeated freezing and thawing.



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## Preparation of MH plates and broth

EUCAST antimicrobial susceptibility testing is based on MH agar and MH broth, without supplements for non-fastidious organisms and with supplements for fastidious organisms (Streptococci, Haemophilus influenzae, Campylobacter and others). The plate and broth for fastidious organisms are named MH-F plates and MH-F broth.

 [Preparation of plates and broth for EUCAST AST.](#) (v 2.0, 2012)

 [Zubereitung der Medien](#) (v 2.0, 2012)

 [Preparación del medio para el estudio de sensibilidad con discos](#) (v 1.1, 2010)

 [Méthodes EUCAST de diffusion en gélose et de détermination des CMI par microdilution en milieu liquide: préparation des milieux.](#) (v 2.0, 2012)

# Reading of *Haemophilus influenzae*



# Reading problems

- Fuzzy zone edges and haze within zone
- Usually due to:
  - Over inoculation
    - Remove excess fluid from the cotton swab
  - Prolonged incubation
  - Humidity
    - Evaluate storage conditions
  - Media



# Problems with media

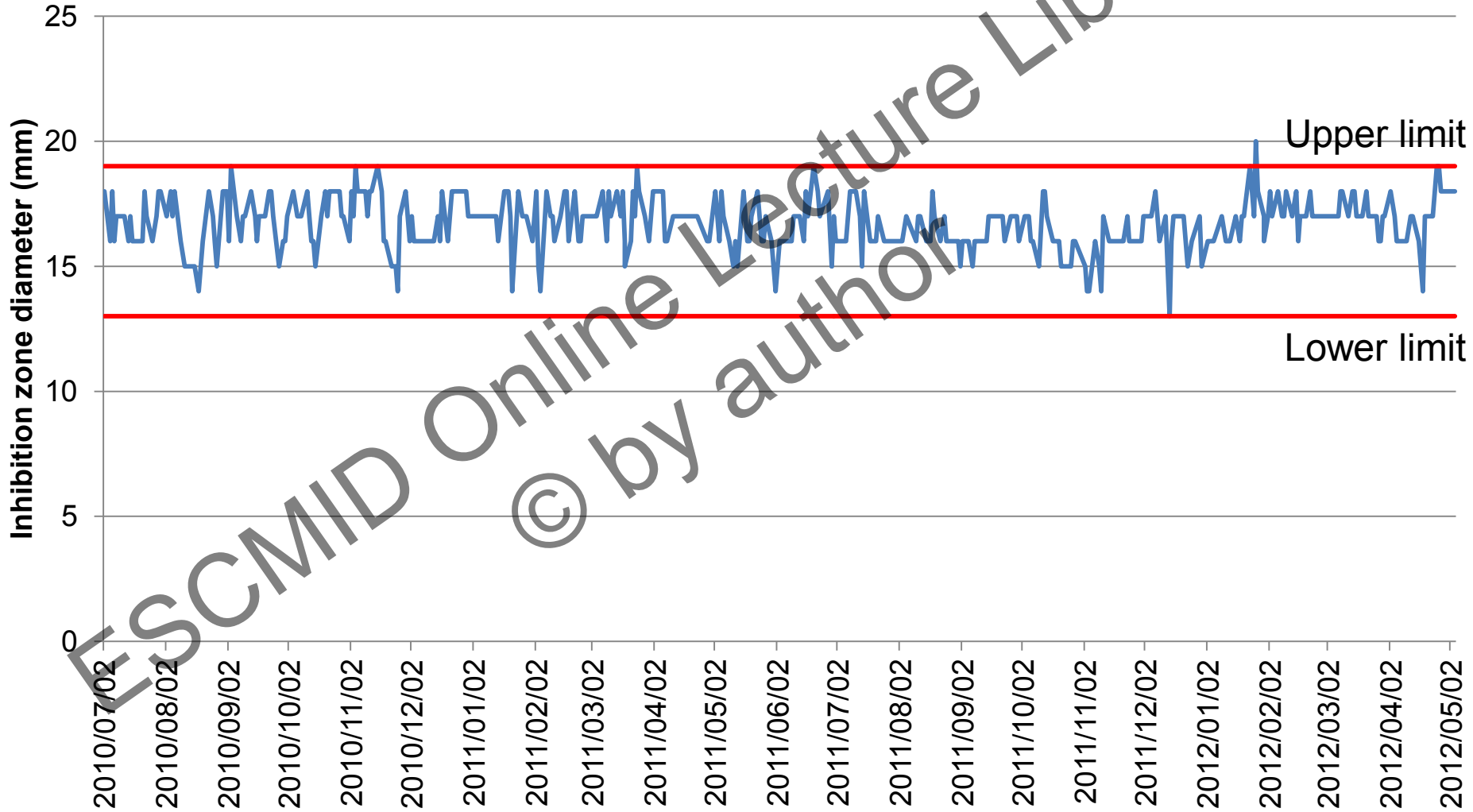


*H. influenzae* with cefotaxime. Haze within zone on the picture to the right.

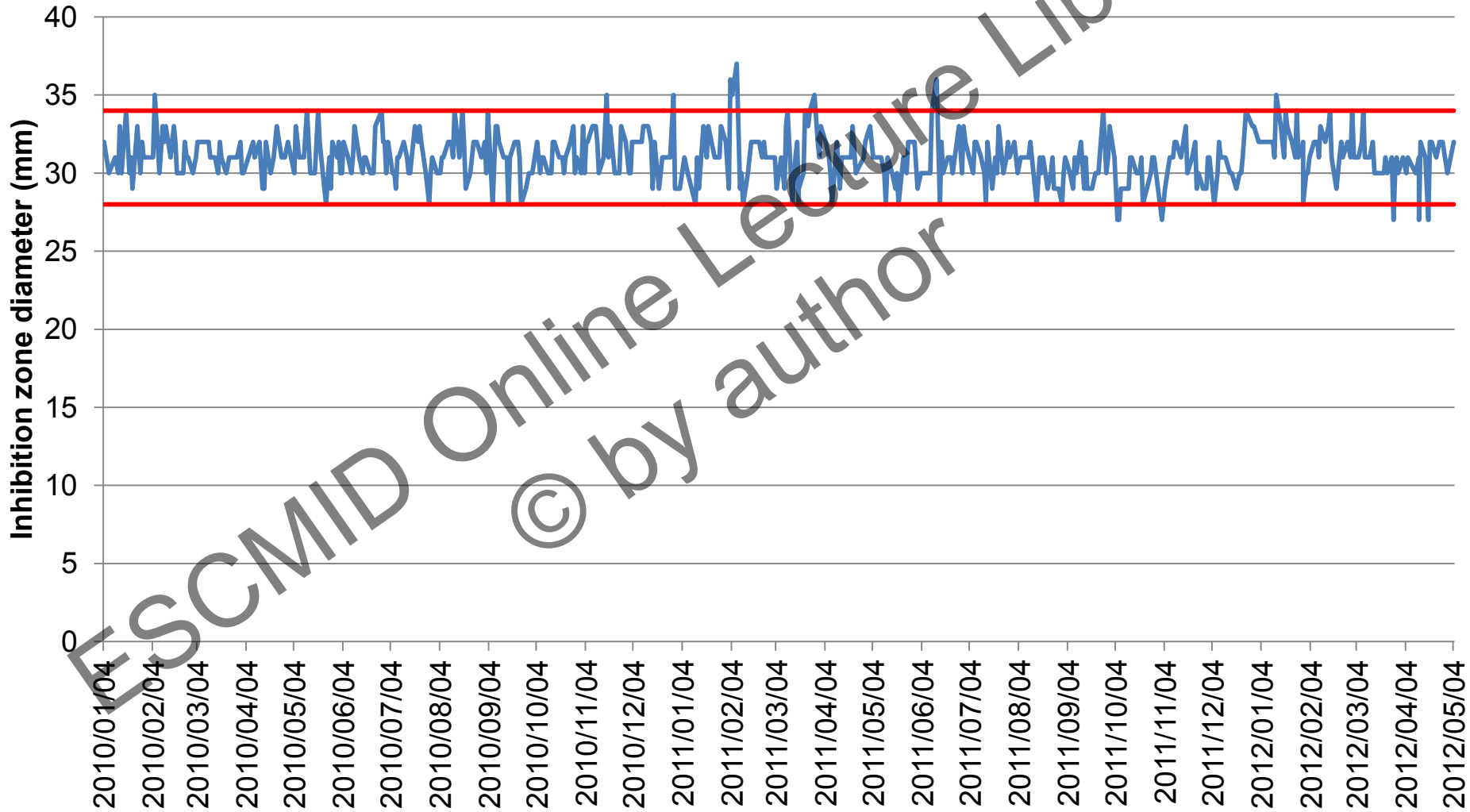
# Reproducibility of MH-F plates

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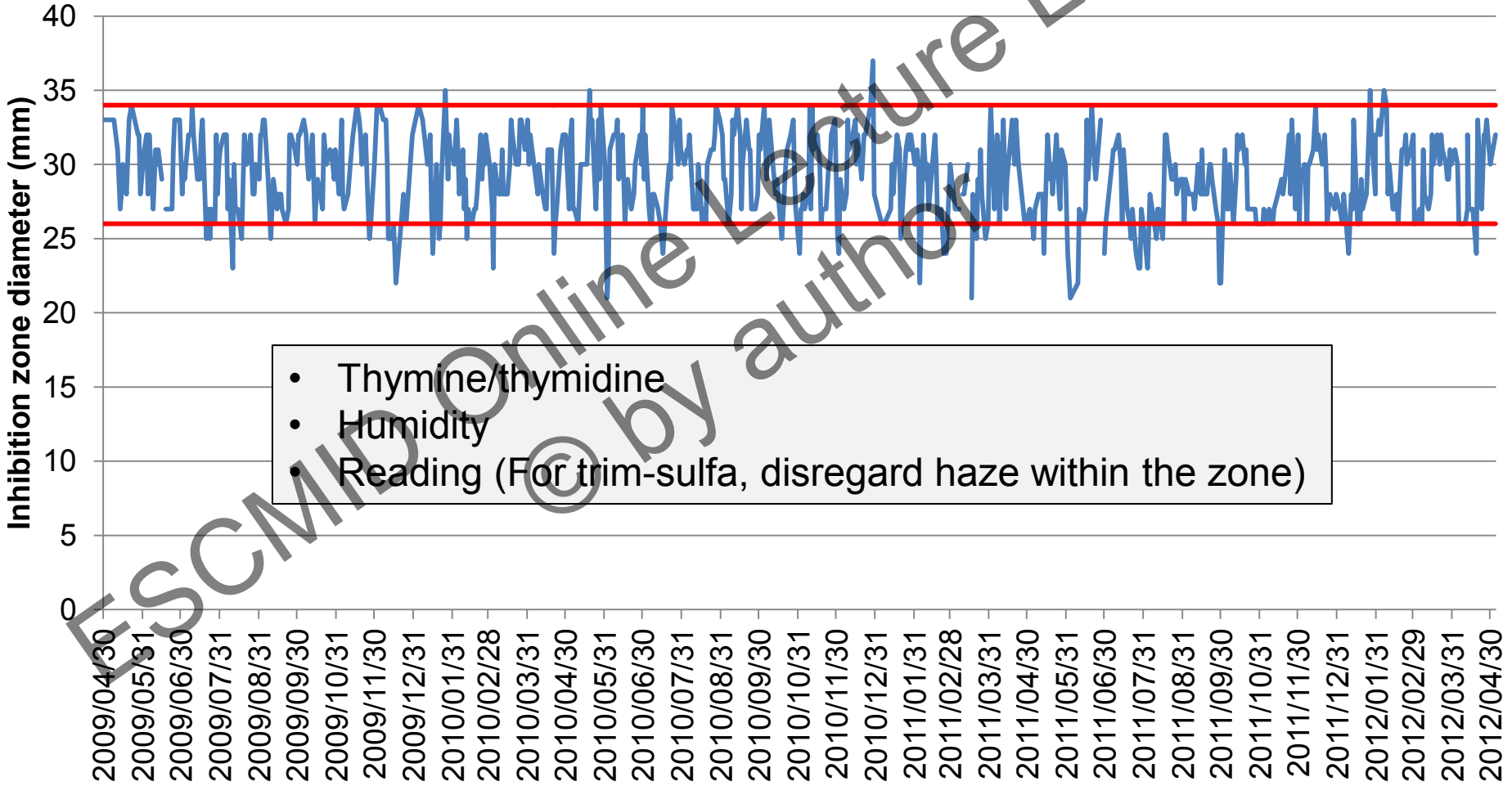
# *H. influenzae* NCTC 8468 with benzylpenicillin 1 unit



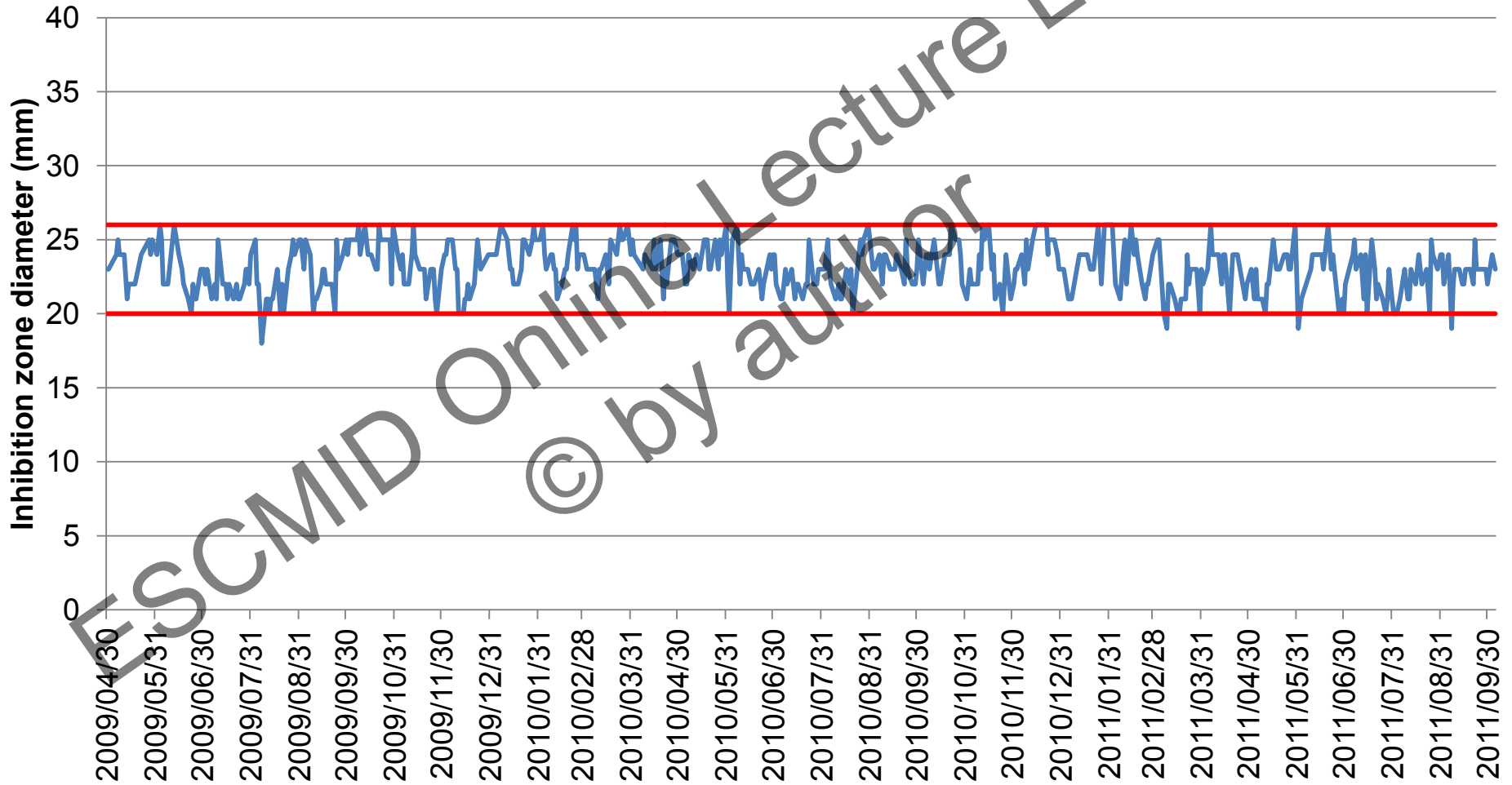
# *H. influenzae* NCTC 8468 with tetracycline 30 µg



***H. influenzae* NCTC 8468  
with trimethoprim-sulfamethoxazole 25 µg**



***S. pneumoniae* ATCC 49619  
with trimethoprim-sulfamethoxazole 25 µg**



# Validation of MH-F broth

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# *H. influenzae* with cefotaxime

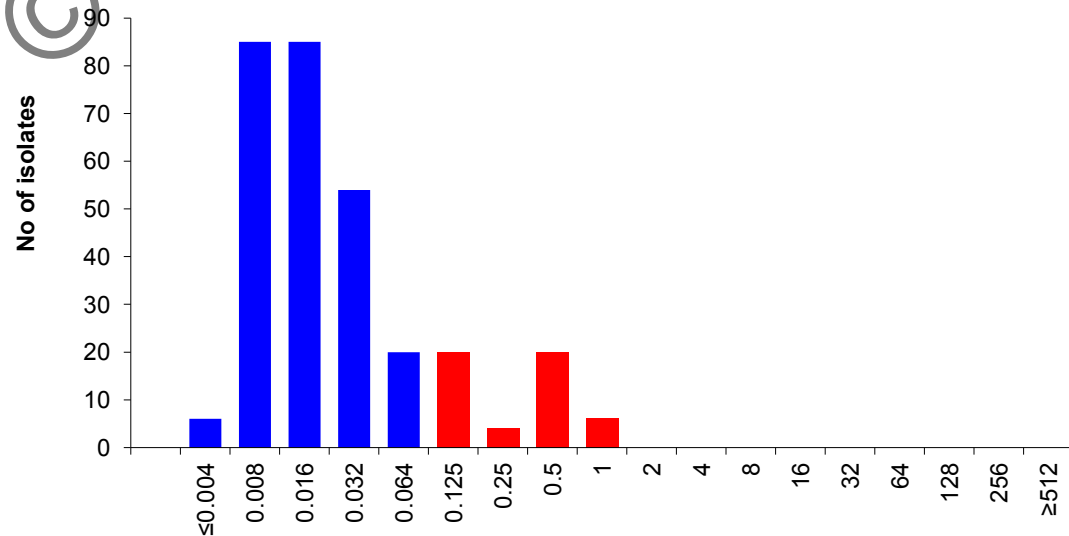
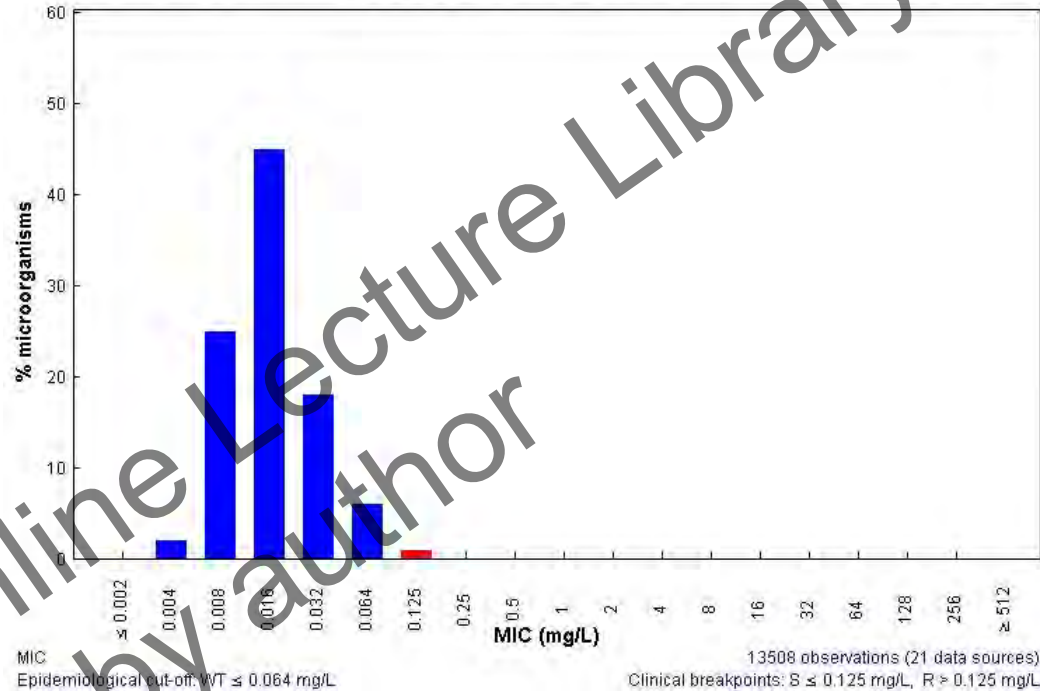
EUCAST database  
13508 observations  
21 data sources

BMD with MH-F broth  
150 isolates tested in  
duplicate

Courtesy of R.N.  
Jones, JMI Labs, US.

Cefotaxime / *Haemophilus influenzae*  
EUCAST MIC Distribution - Reference Database 2010-10-08

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





# *H. influenzae* with ciprofloxacin

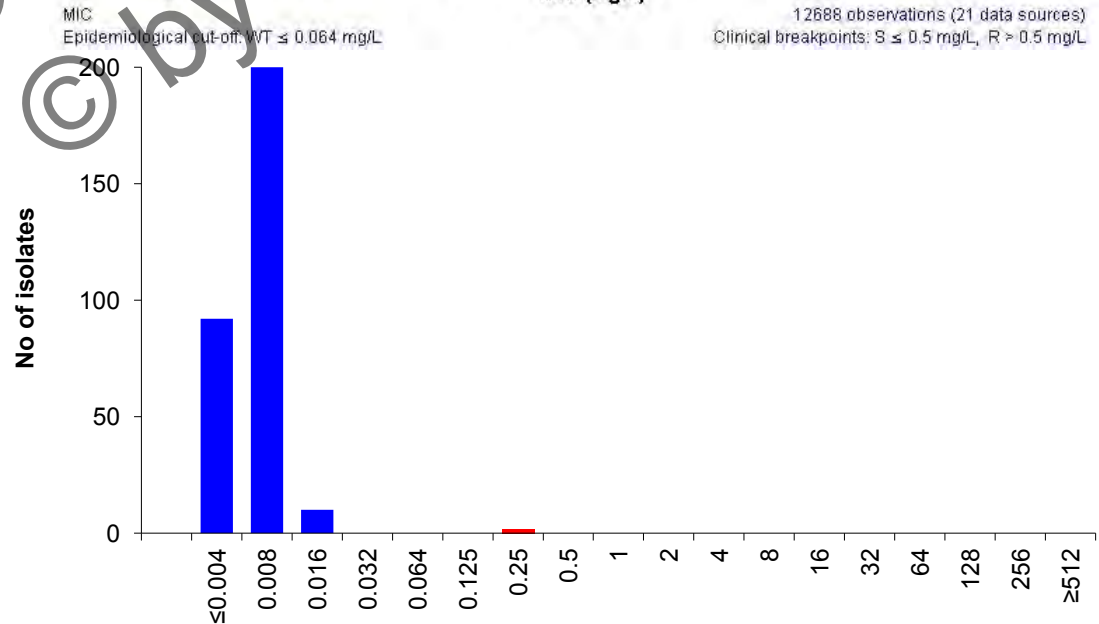
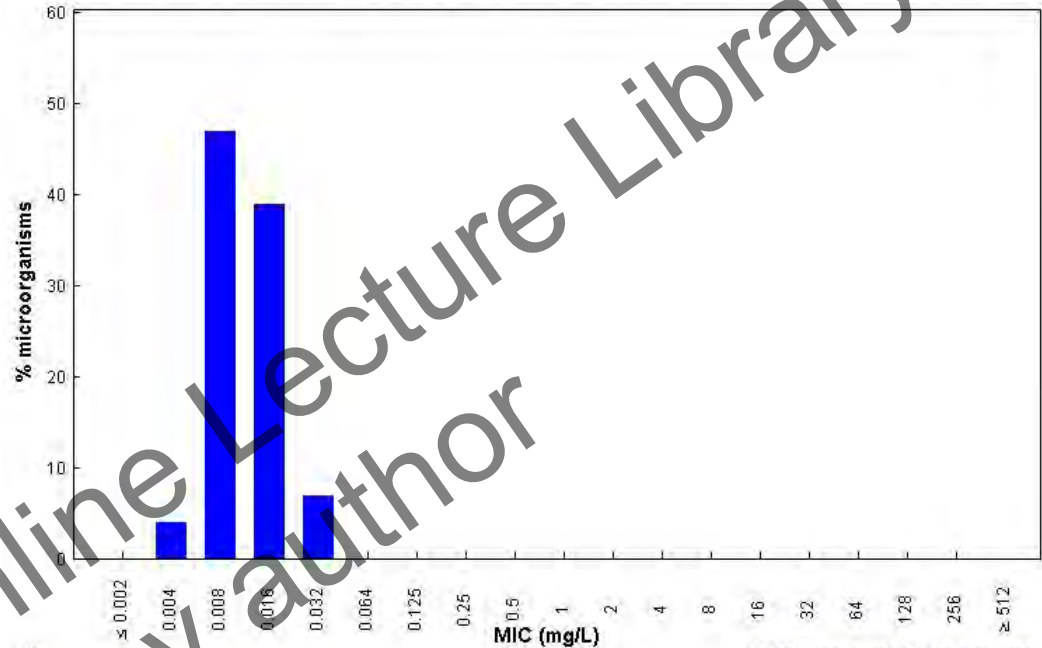
EUCAST database  
12688 observations  
21 data sources

BMD with MH-F broth  
150 isolates tested in  
duplicate

Courtesy of R.N.  
Jones, JMI Labs, US.

Ciprofloxacin / *Haemophilus influenzae*  
EUCAST MIC Distribution - Reference Database 2010-09-03

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  $\le 0.064$  mg/L

12688 observations (21 data sources)  
Clinical breakpoints: S  $\le 0.5$  mg/L, R  $> 0.5$  mg/L

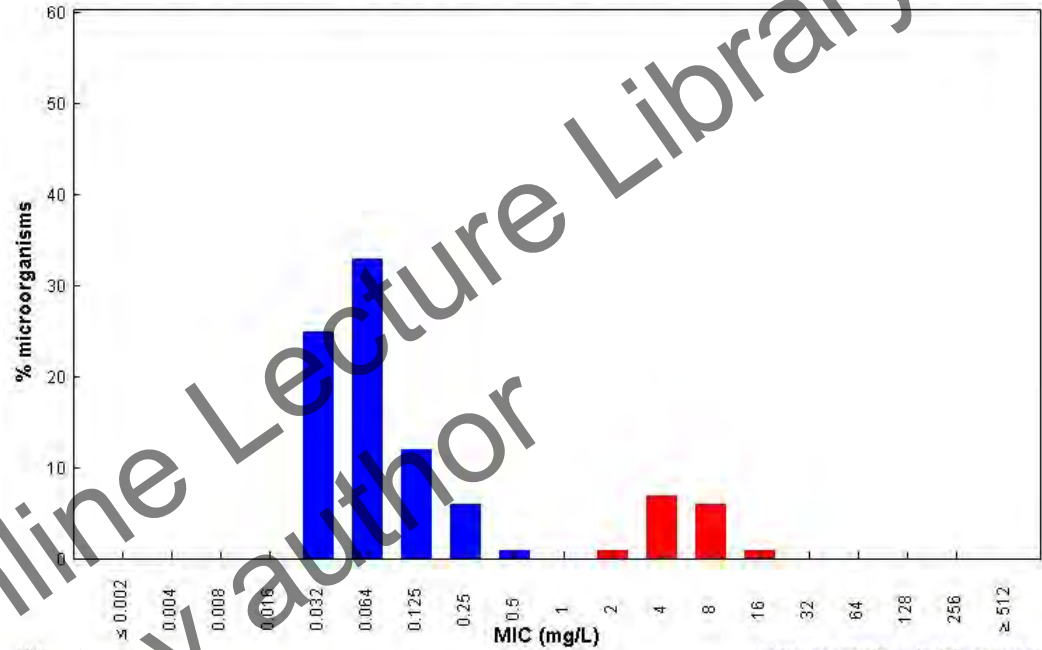
# *H. influenzae* with trimethoprim- sulfamethoxazole

EUCAST database  
21371 observations  
6 data sources

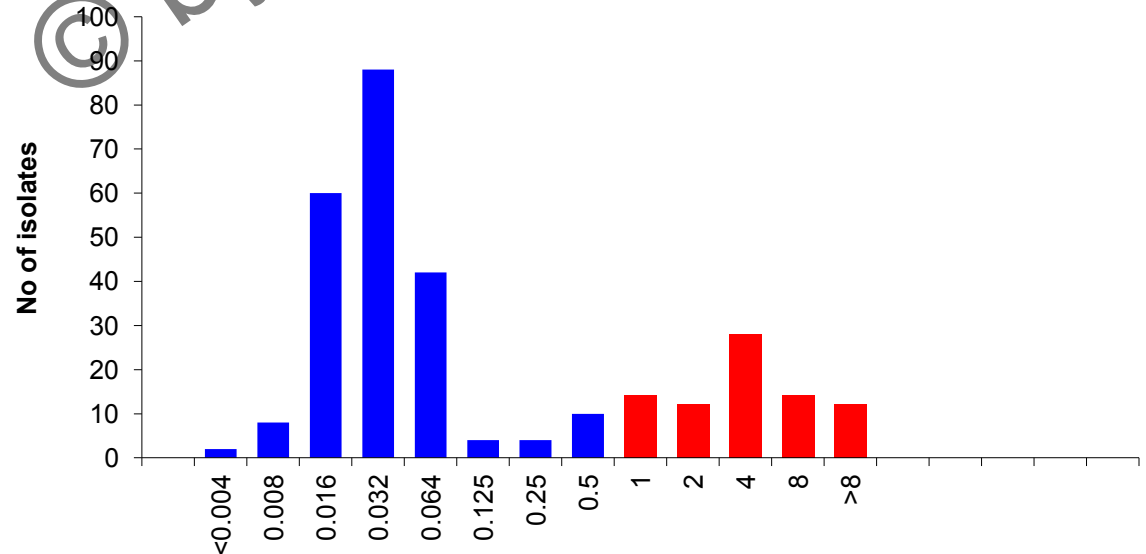
BMD with MH-F broth  
150 isolates tested in  
duplicate

Courtesy of R.N.  
Jones, JMI Labs, US.

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  
21371 observations (6 data sources)  
Clinical breakpoints: S ≤ 0.5 mg/L, R ≥ 1 mg/L



# Clinical breakpoints

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# Breakpoints differ!

Antimicrobial agent	EUCAST <sup>1</sup>		CLSI <sup>2</sup>	
	S ≤	R >	S ≤	R >
Ampicillin	1	1	1	2
Amoxicillin-clavulanic acid	2	2	4	4
Cefotaxime/Ceftriaxone	0.12	0.12	2	-
Meropenem			0.5	-
- non-meningitis	2	2		
- meningitis	0.25	1		
Ciprofloxacin	0.5	0.5	1	-
Chloramphenicol	2	2	2	4
Trimethoprim-sulfamethoxazole	0.5	1	0.5	2

1. EUCAST Clinical Breakpoint Table v. 2.0, 2012.
2. Performance Standards for AST, M100-S22, Vol 32 No 3, 2012. Breakpoints expressed with EUCAST terminology.

# Validation of EUCAST zone diameter breakpoints for *H. influenzae*

- 150 clinical isolates from the SENTRY collection
  - BMD with MH-F broth at JMI, US
  - Disk diffusion and PCR for PBP mutations at EUCAST Laboratory for AST
  - MIC/zone diameter correlates for all agents with zone diameter breakpoints
- Adjusted breakpoints presented in/at:
  - EUCAST breakpoint table v 2.0
  - ECCMID 2012

**Table 1. EUCAST clinical breakpoints for *Haemophilus influenzae*.**

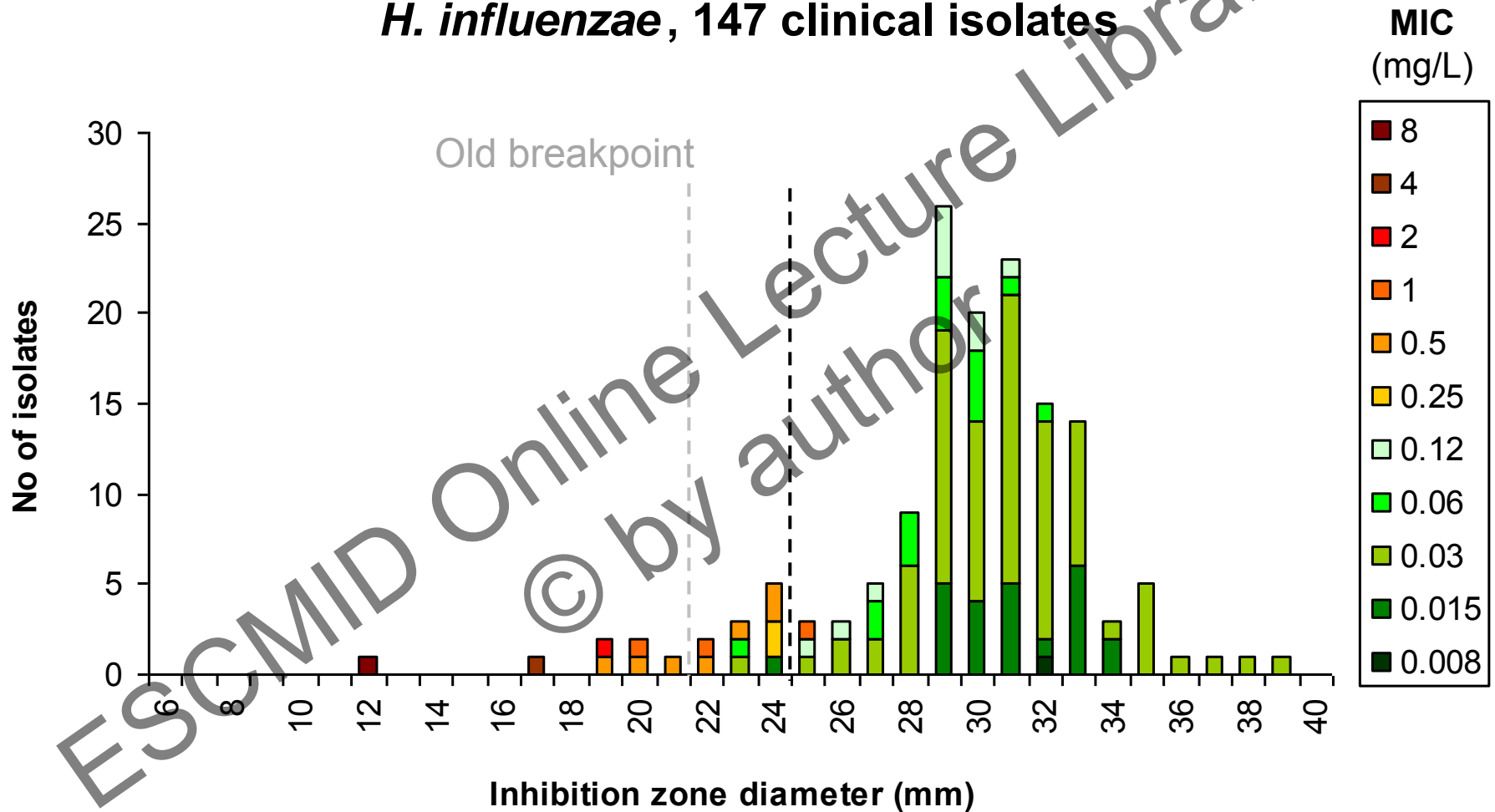
EUCAST Clinical Breakpoint Table v. 2.0, January 2012.

New breakpoints highlighted in yellow, with old breakpoints in parenthesis.

Antimicrobial agent	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Ampicillin	1	1	2	16	16
Amoxicillin-clavulanate	2 (1)	2 (1)	2-1	17 (20*)	17 (20*)
Cefepime	0.25	0.25	30	27 (25)	27 (25)
Cefixime	0.12	0.12	5	25 (22)	25 (22)
Cefotaxime	0.12	0.12	5	26 (22)	26 (22)
Cefpodoxime	0.25	0.5	10	26 (24)	23 (21)
Ceftibuten	1	1	30	25 (24)	25 (24)
Ceftriaxone	0.12	0.12	30	30 (27)	30 (27)
Cefuroxime	1	2	30	26 (25)	25 (22)
Cefuroxime axetil	0.12	1	30	50	26 (25)
Doripenem	1	1	10	20	20
Ertapenem	0.5	0.5	10	20	20
Imipenem	2	2	10	20 (16)	20 (16)
Meropenem (non-meningitis)	2	2	10	20	20
Ciprofloxacin	0.5	0.5	5	26 (23)	26 (23)
Levofloxacin	1	1	5	26 (21)	26 (21)
Moxifloxacin	0.5	0.5	5	25 (23)	25 (23)
Ofloxacin	0.5	0.5	5	23 (21)	23 (21)
Erythromycin	0.5	16	15	50	10 (12)
Telithromycin	0.12	8	15	50	12 (14)
Minocycline	1	2	30	24 (25)	21 (22)
Tetracycline	1	2	30	25 (24)	22 (21)
Chloramphenicol	2 (1)	2	30	28	28 (25)
Rifampicin	1 (0.5)	1 (0.5)	5	18	18
Trimethoprim-sulfamethoxazole	0.5	1	1.25-23.75	23	20

\* Old zone diameter breakpoint for amoxicillin-clavulanate 20-10 µg.

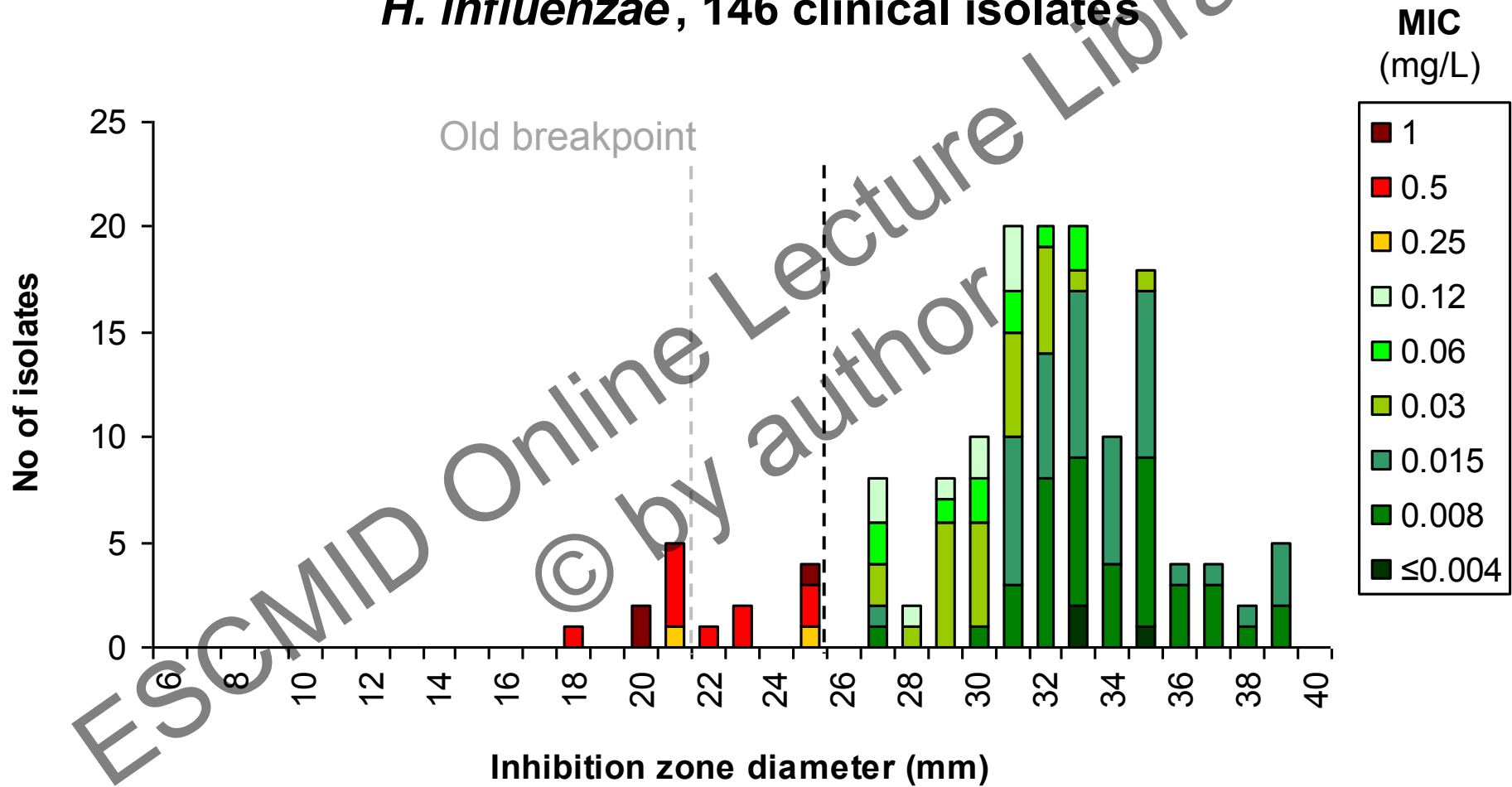
## Cefixime 5 $\mu$ g vs. MIC *H. influenzae*, 147 clinical isolates



Breakpoints		ECOFF
MIC	S $\leq$ 0.12, R>0.12 mg/L	WT $\leq$ 0.12 mg/L
Zone diameter	S $\geq$ 25, R<25 mm	

# Cefotaxime 5 µg vs. MIC

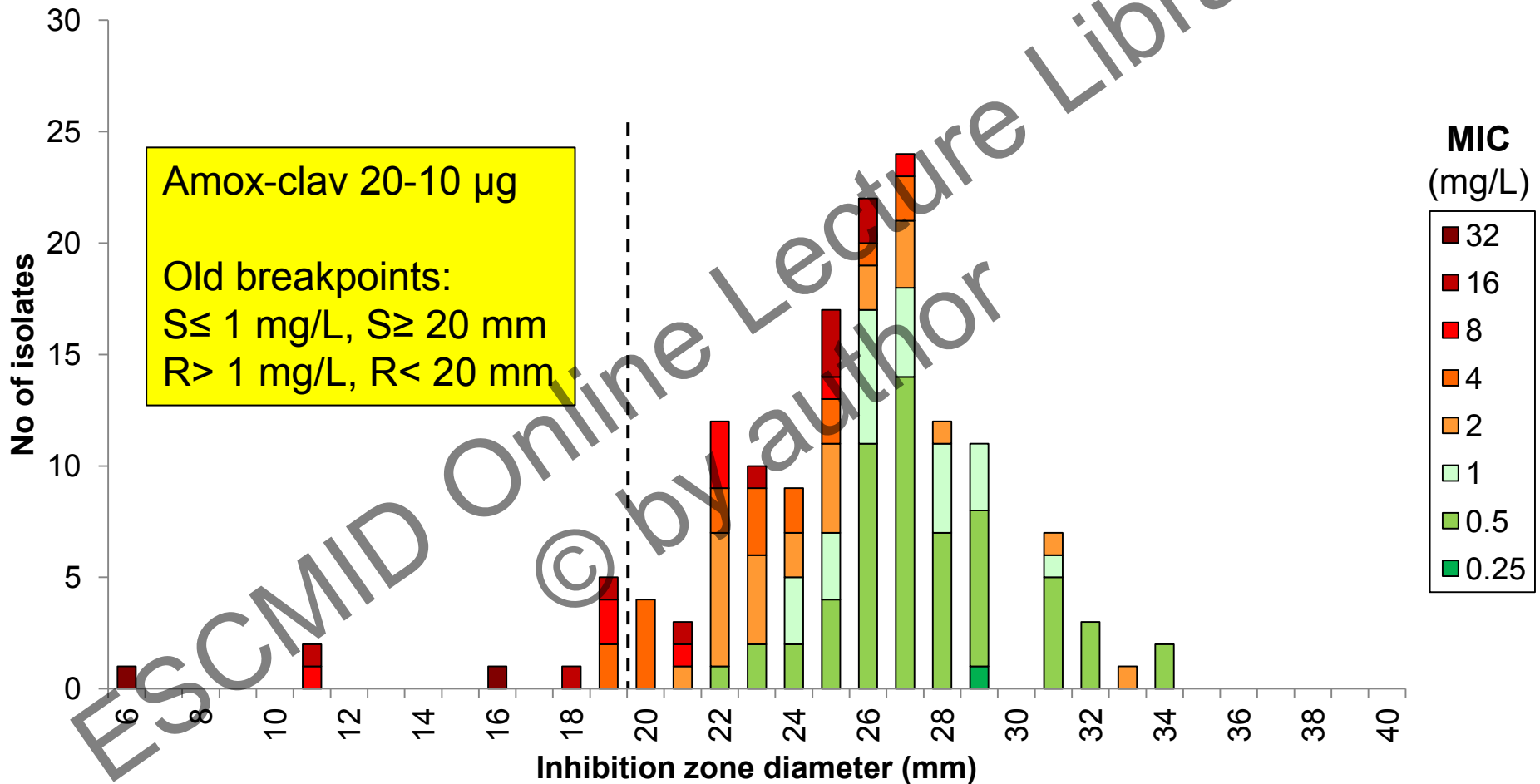
## *H. influenzae*, 146 clinical isolates



Breakpoints		ECOFF
MIC	S ≤ 0.12, R > 0.12 mg/L	WT ≤ 0.06 mg/L
Zone diameter	S ≥ 26, R < 26 mm	

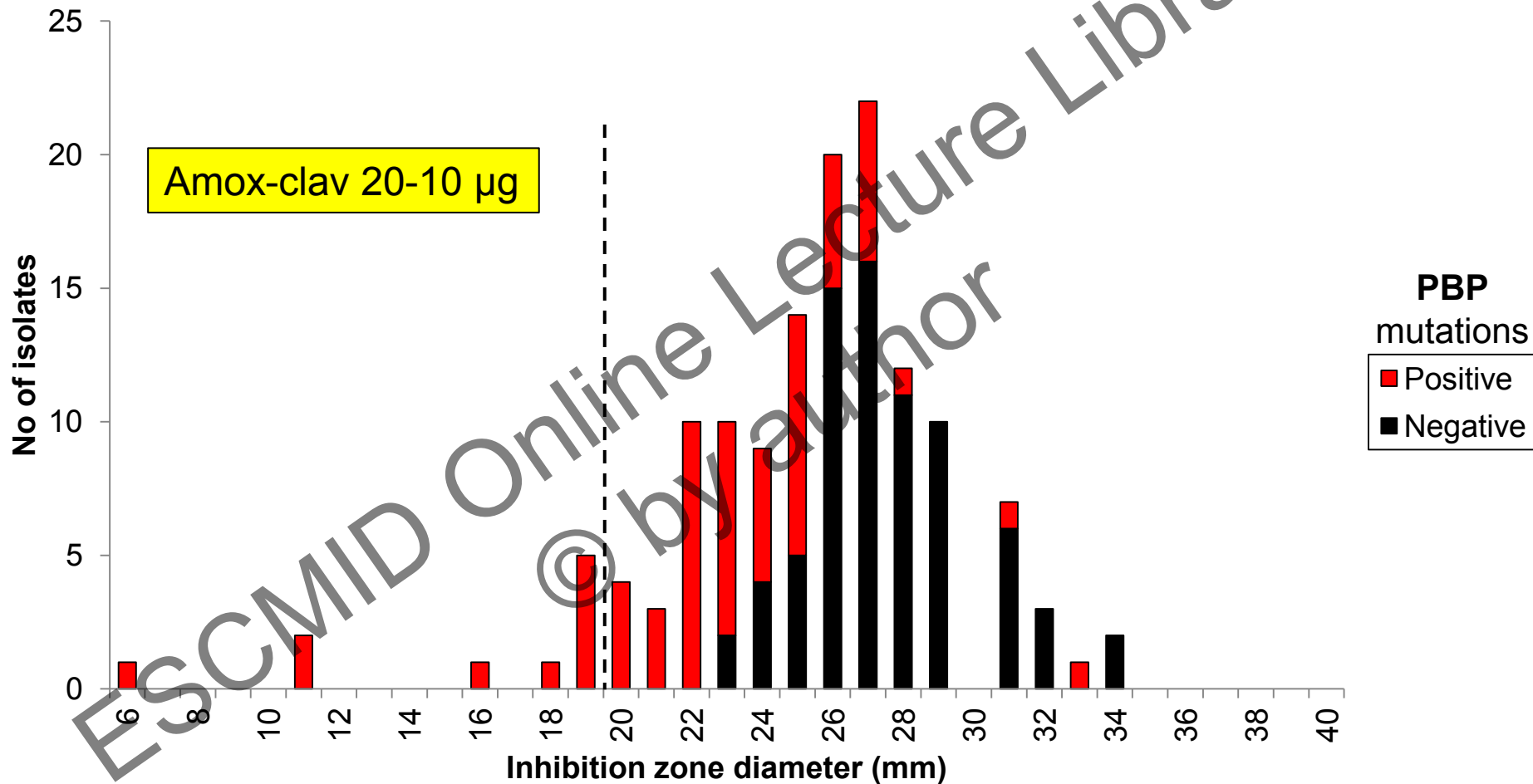


# Amoxicillin-clavulanate 20-10 µg vs. MIC *H. influenzae*, 147 clinical isolates



Breakpoints	
MIC	$S \leq 1$ , $R > 1 \text{ mg/L}$
Zone diameter	$S \geq 20$ , $R < 20 \text{ mm}$

# Amoxicillin-clavulanate 20-10 µg vs. PBP mutations *H. influenzae*, 137 clinical isolates

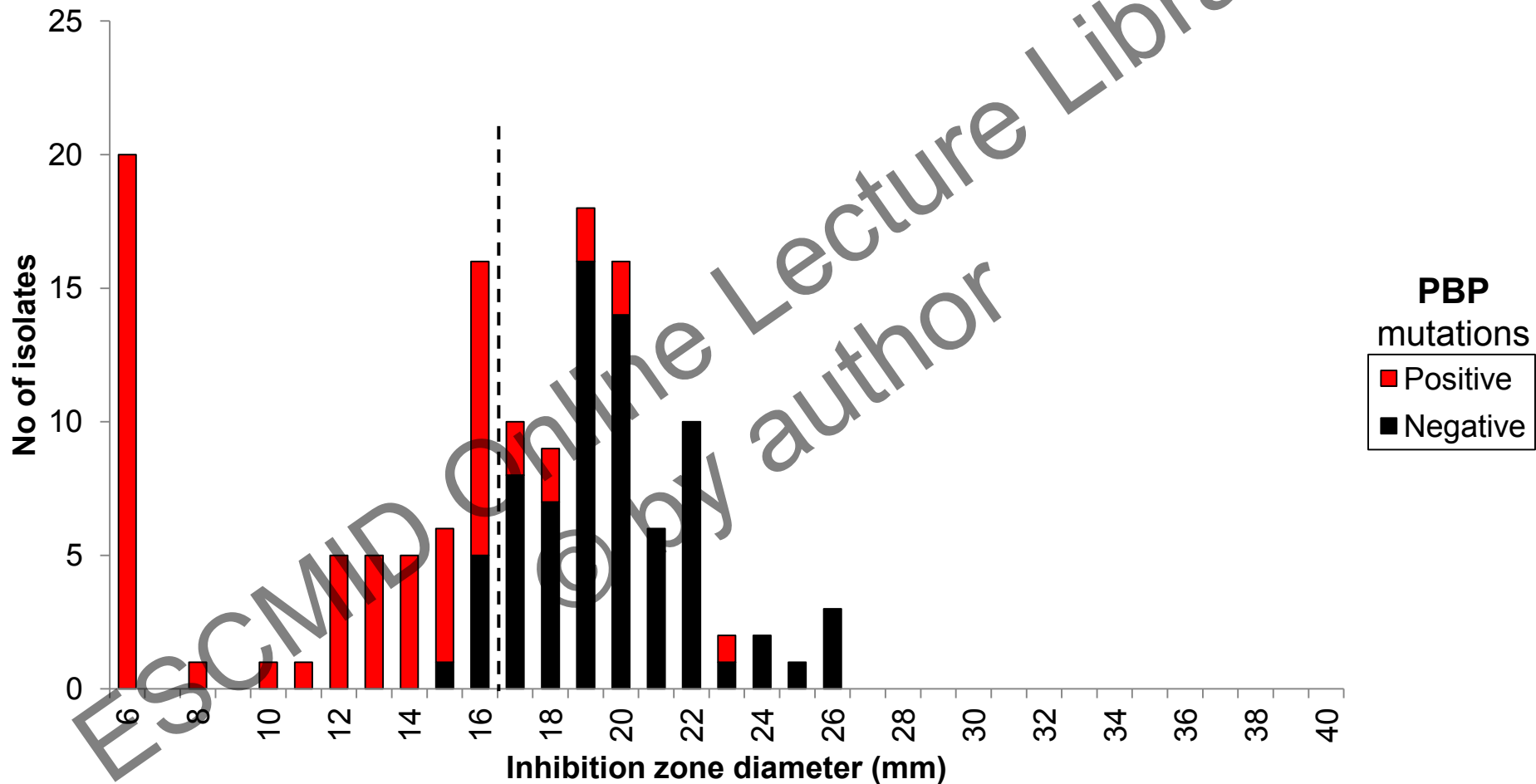


## Breakpoints

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

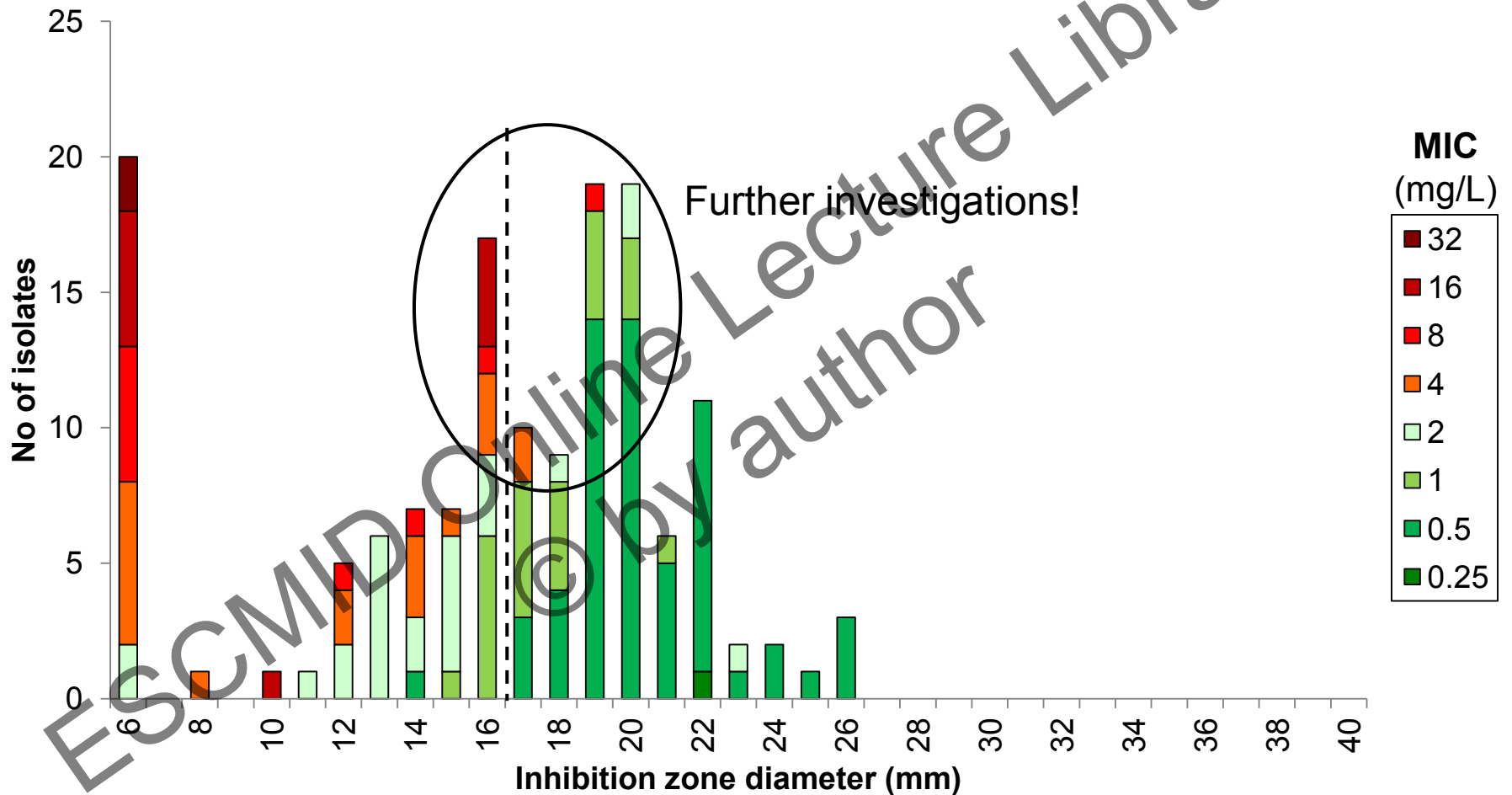
Zone diameter  $S \geq 20, R < 20$  mm

# Amoxicillin-clavulanate 2-1 µg vs. PBP mutations *H. influenzae*, 137 clinical isolates



<b>Breakpoints</b>	
MIC	S ≤ 2, R > 2 mg/L
Zone diameter	S ≥ 17, R < 17 mm

# Amoxicillin-clavulanate 2-1 µg vs. MIC *H. influenzae*, 147 clinical isolates



Breakpoints	
MIC	S ≤ 2, R > 2 mg/L
Zone diameter	S ≥ 17, R < 17 mm

# Screening for beta-lactam resistance with benzylpenicillin 1 unit

- Benzylpenicillin (PCG) 1 unit detects:
  - Beta-lactamase
  - Ampicillin resistance
  - Other beta-lactam resistance

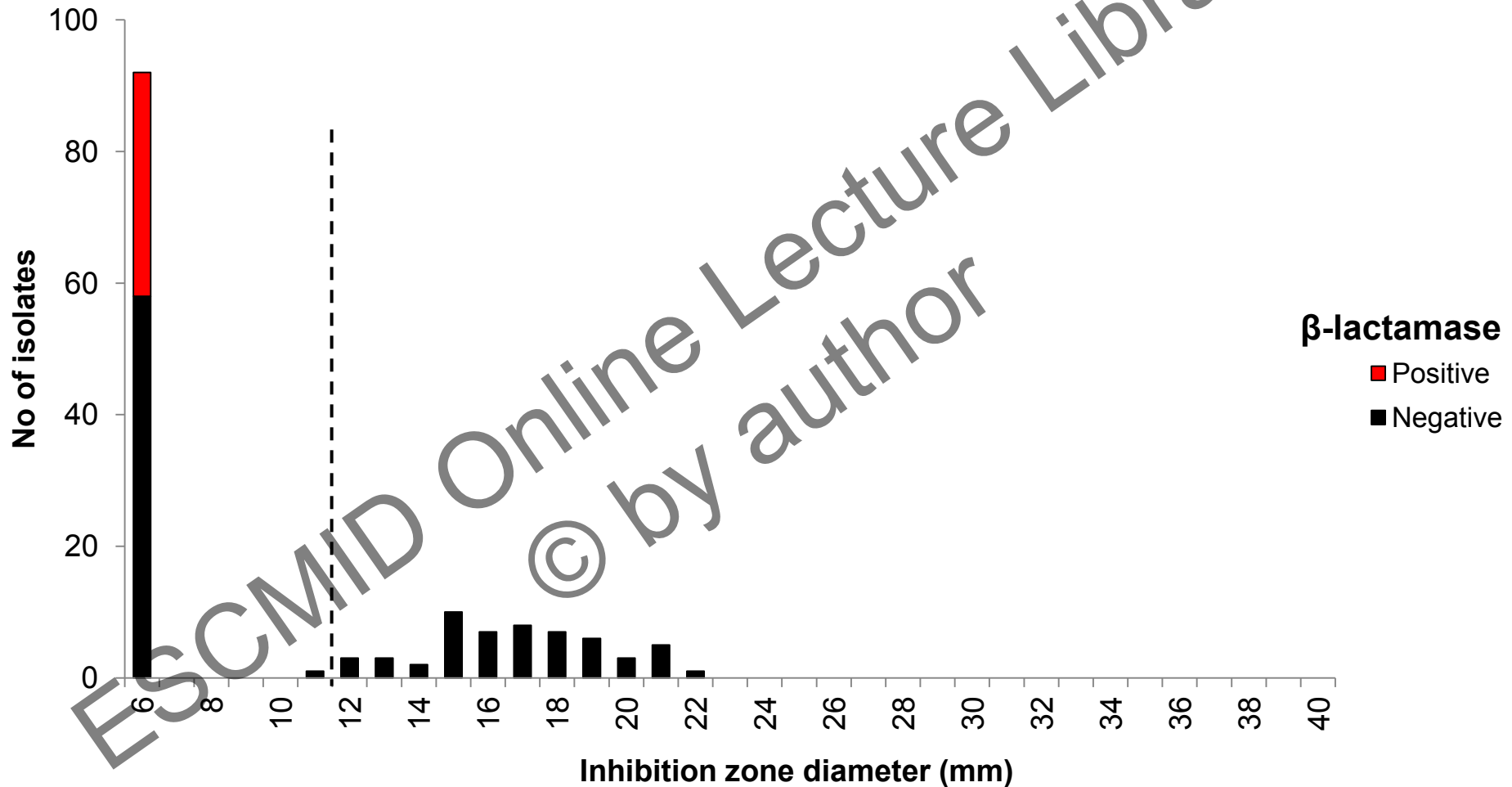
PCG 1 unit  $\geq 12$  mm

Report susceptible to all betalactams with clinical breakpoints (including those with "Note")

PCG 1 unit  $< 12$  mm

Check for beta-lactamase and test each agent to report

# Benzylpenicillin 1 unit vs. $\beta$ -lactamase *H. influenzae*, 148 clinical isolates

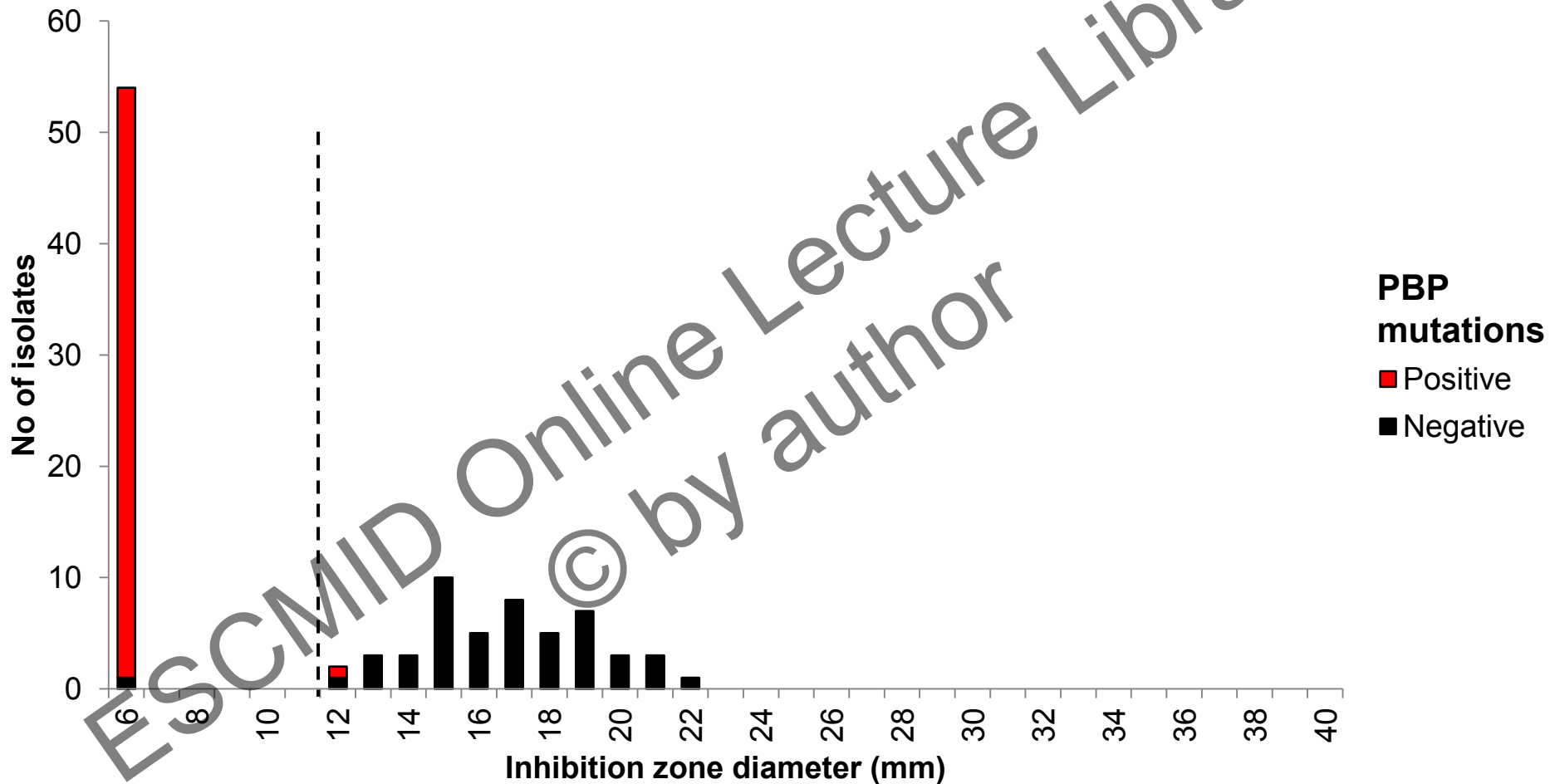


## Breakpoints

Benzylpenicillin zone diameter (screen)

$S \geq 12$ ,  $R < 12$  mm

# Benzylpenicillin 1 unit vs. PBP mutations *H. influenzae*, 104 $\beta$ -lactamase negative clinical isolates

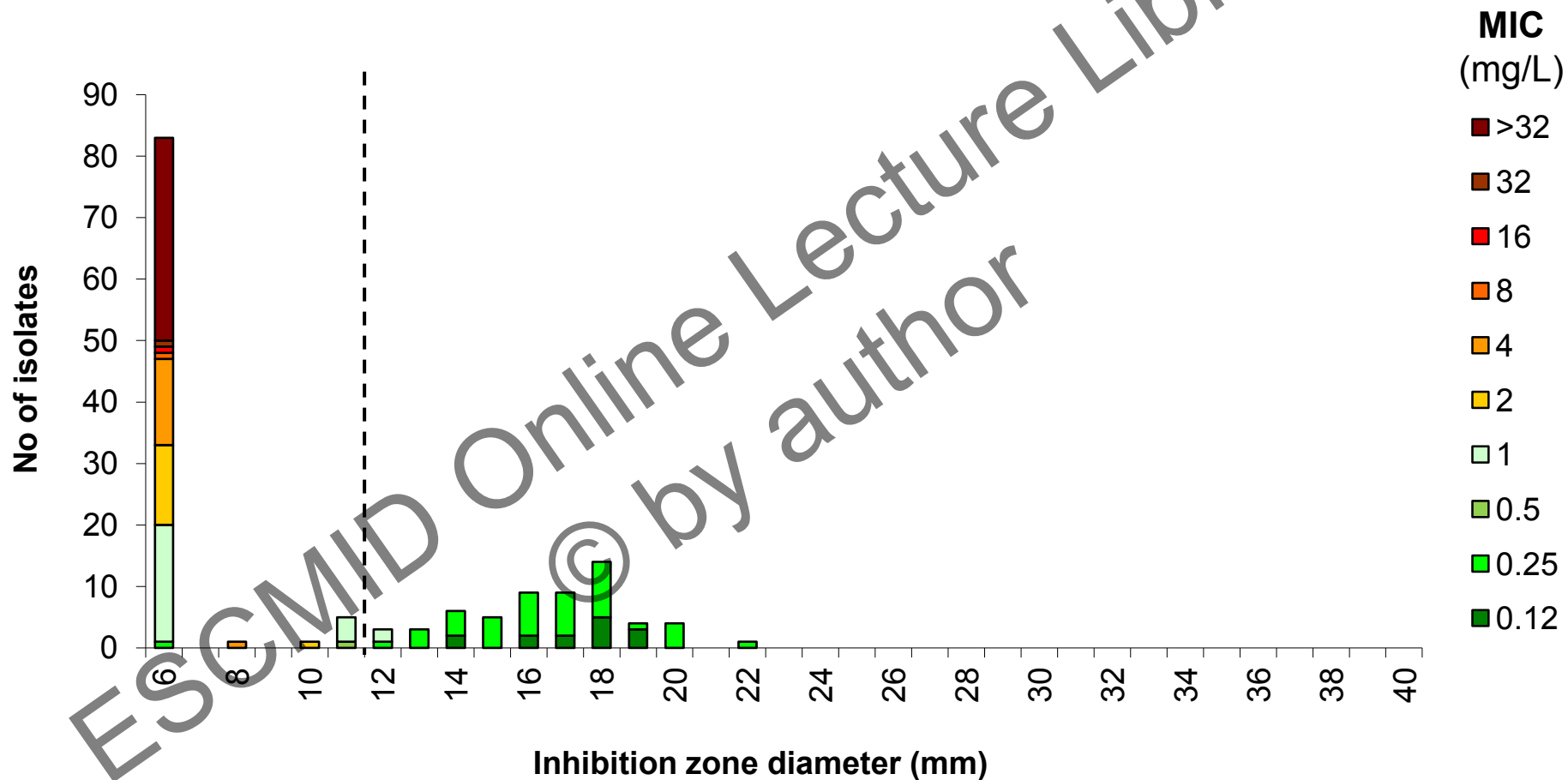


## Breakpoints

Benzylpenicillin zone diameter (screen)

$S \geq 12$ ,  $R < 12$  mm

## Benzylpenicillin 1 unit vs. Ampicillin MIC *H. influenzae*, 148 clinical isolates



### Breakpoints

Ampicillin MIC

Benzylpenicillin zone diameter (screen)

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

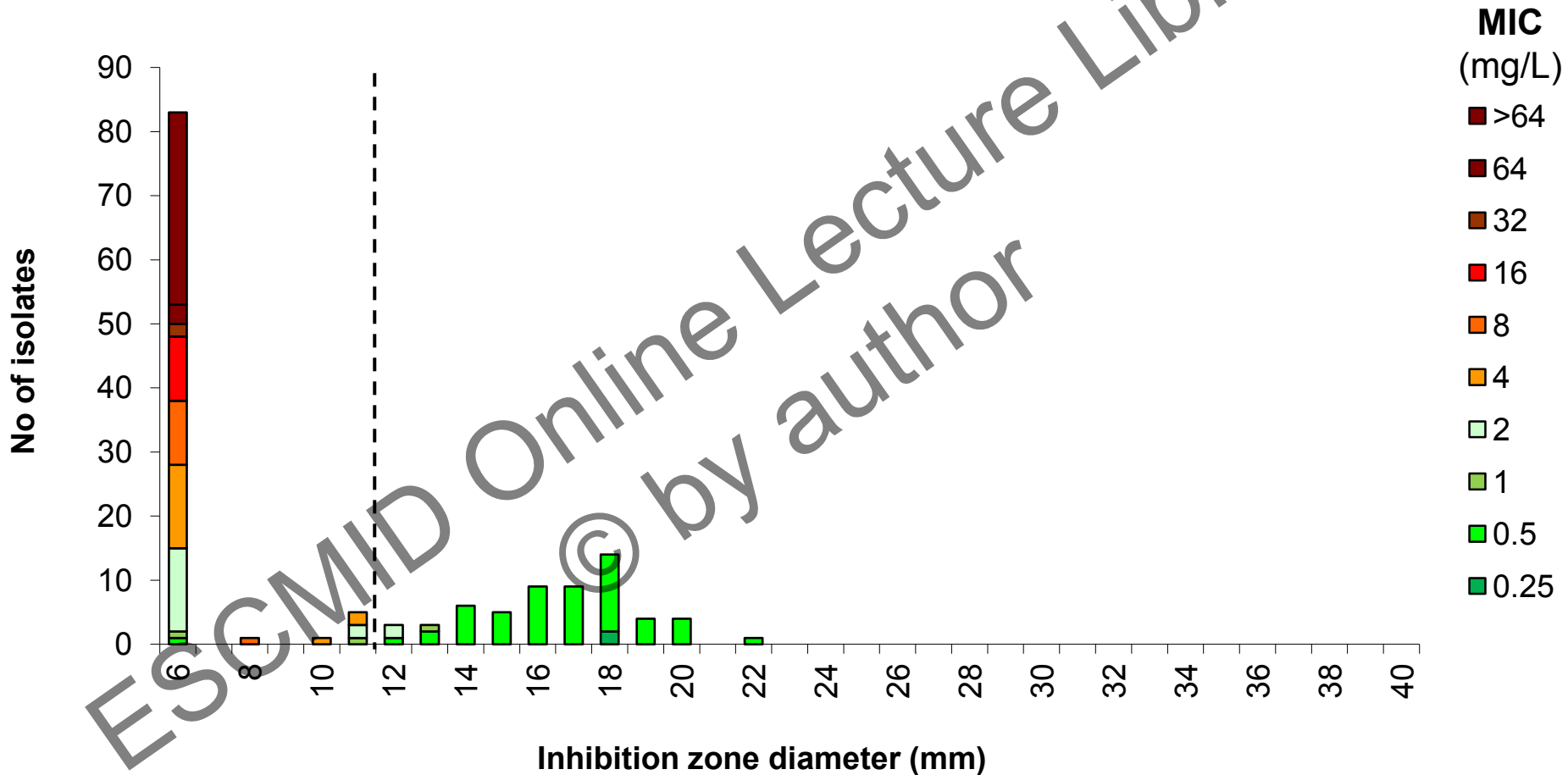
$S \geq 12$ ,  $R < 12$  mm

### ECOFF

$WT \leq 1$  mg/L



## Benzylpenicillin 1 unit vs. Amoxicillin MIC *H. influenzae*, 148 clinical isolates



### Breakpoints

Amoxicillin MIC

Benzylpenicillin zone diameter (screen)

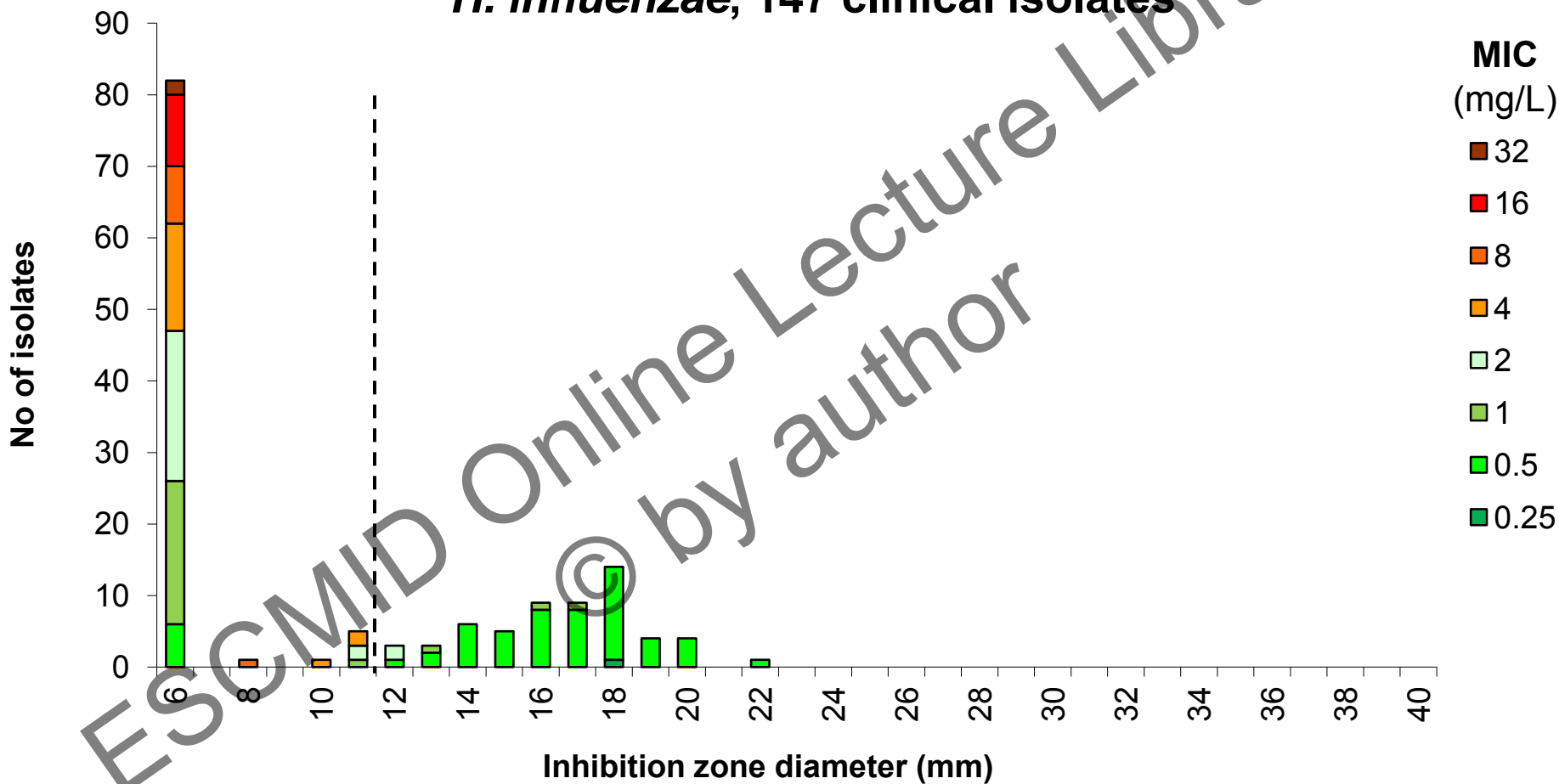
$S \leq 2, R > 2$  mg/L

$S \geq 12, R < 12$  mm

### ECOFF

WT  $\leq 2$  mg/L

## Benzylpenicillin 1 unit vs. Amoxicillin-clavulanate MIC *H. influenzae*, 147 clinical isolates



### Breakpoints

Amoxicillin-clavulanate MIC

Benzylpenicillin zone diameter (screen)

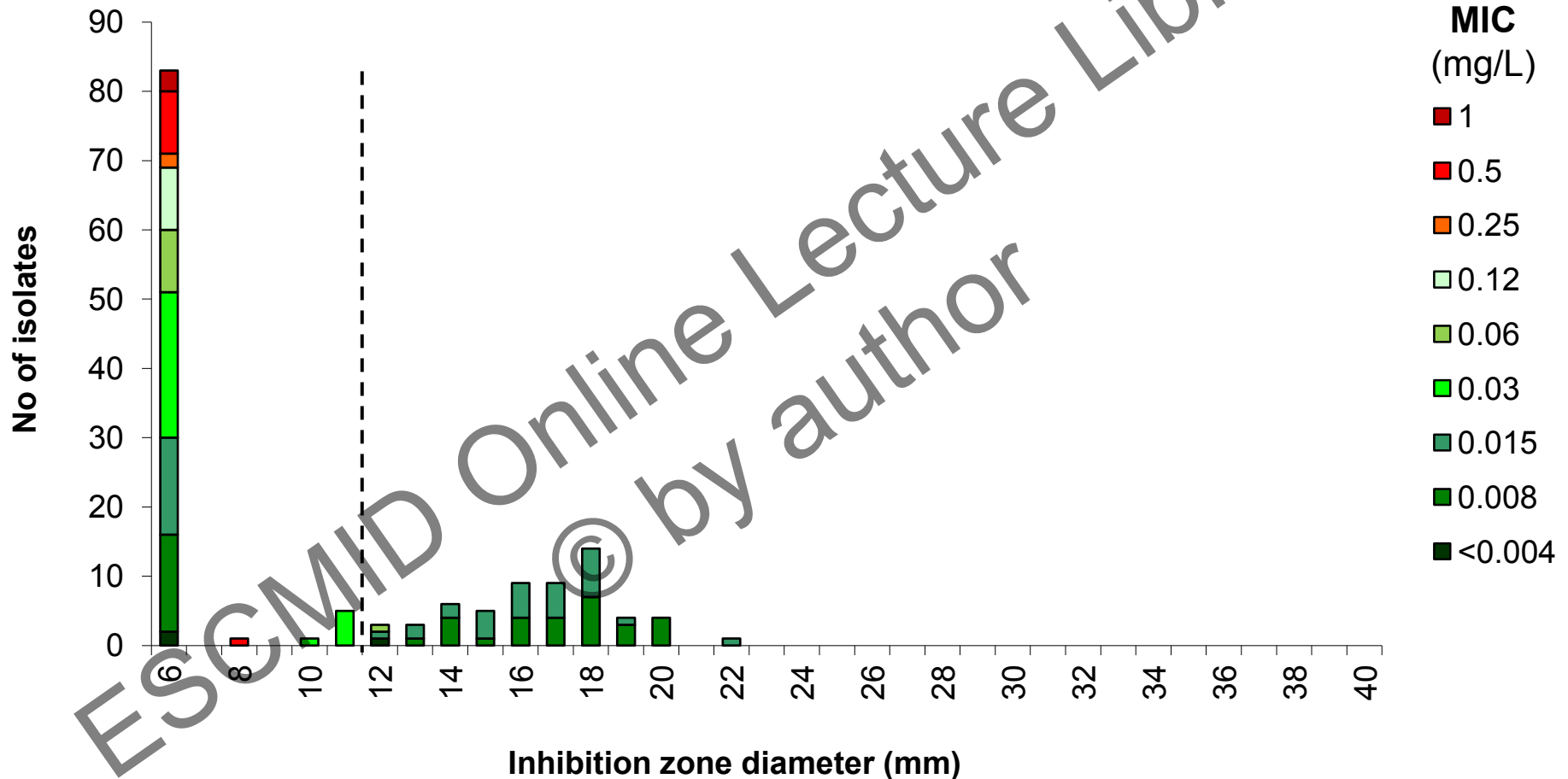
$S \leq 2$ ,  $R > 2$  mg/L

$S \geq 12$ ,  $R < 12$  mm

### ECOFF

$WT \leq 2$  mg/L

## Benzylpenicillin 1 unit vs. Cefotaxime MIC *H. influenzae*, 148 clinical isolates



### Breakpoints

Cefotaxime MIC

Benzylpenicillin zone diameter (screen)

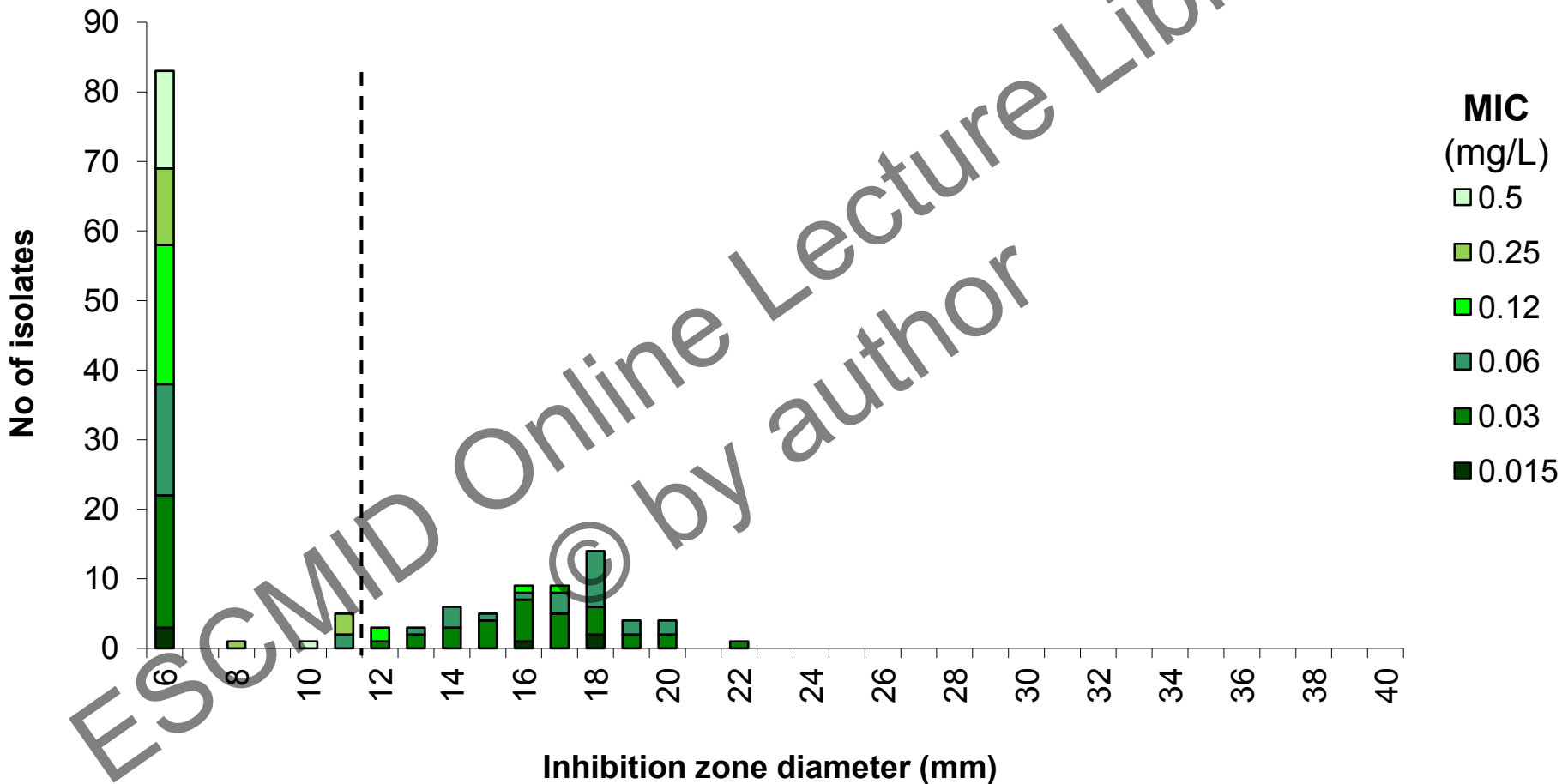
$S \leq 0.12$ ,  $R > 0.12$  mg/L

$S \geq 12$ ,  $R < 12$  mm

### ECOFF

WT  $\leq 0.06$  mg/L

## Benzylpenicillin 1 unit vs. Meropenem MIC *H. influenzae*, 148 clinical isolates



### Breakpoints

Meropenem MIC

Benzylpenicillin zone diameter (screen)

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

$S \geq 12$ ,  $R < 12$  mm

### ECOFF

$WT \leq 0.25$  mg/L



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