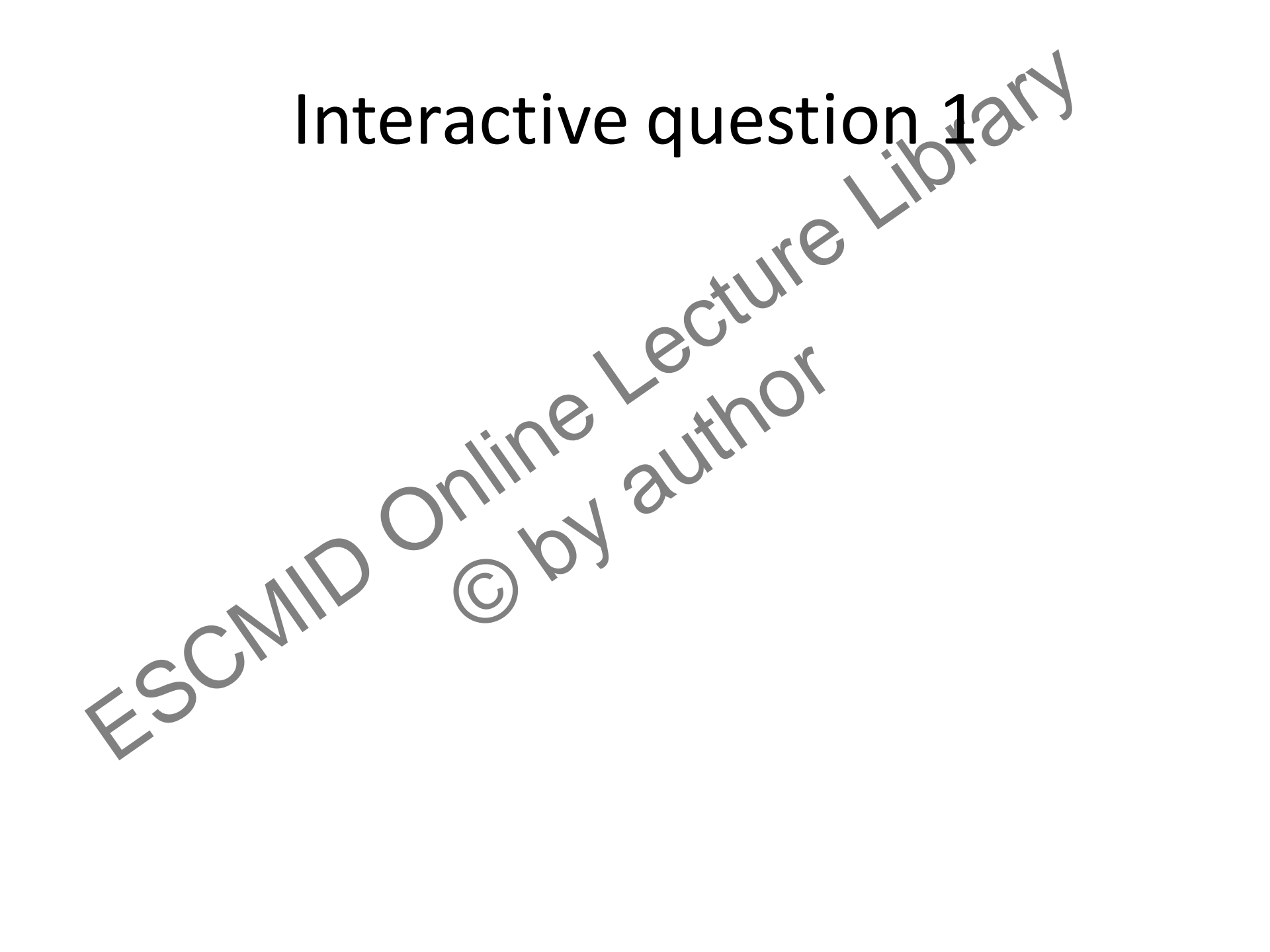


What's new in EUCAST methods?

Derek Brown

EUCAST Scientific Secretary

Interactive question 1



MIC determination

- MH-F broth for broth microdilution testing of fastidious microorganisms
- Gradient MIC tests on MH-F agar

Validation of MH-F broth

(MH broth with 5% lysed horse blood and 20 mg/L β -NAD)

- Comparison of distributions of MICs with MH-F broth with collated distributions on EUCAST MIC distribution website (<http://mic.eucast.org/Eucast2/>)
- Tests on *H. influenzae* and *S. pneumoniae*
- MH-F data courtesy of Ron Jones, JMI Laboratories, USA

Validation of MH-F broth

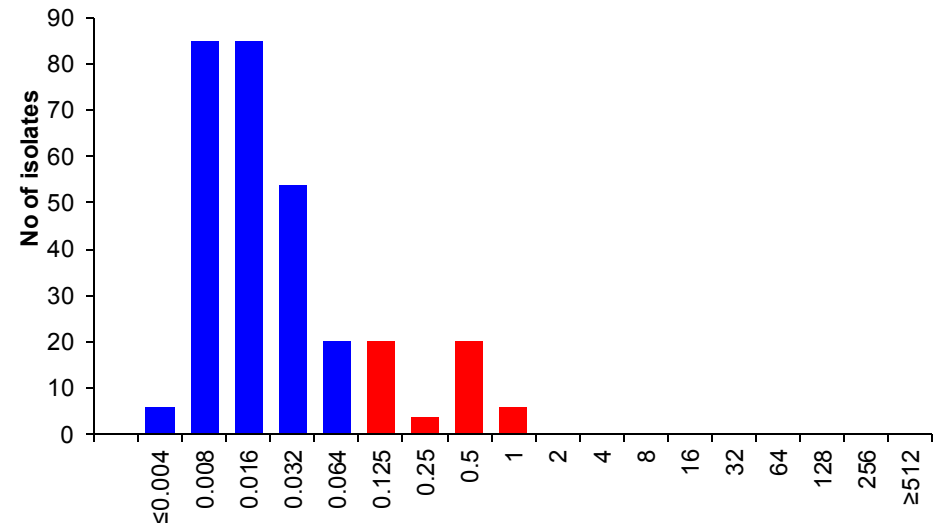
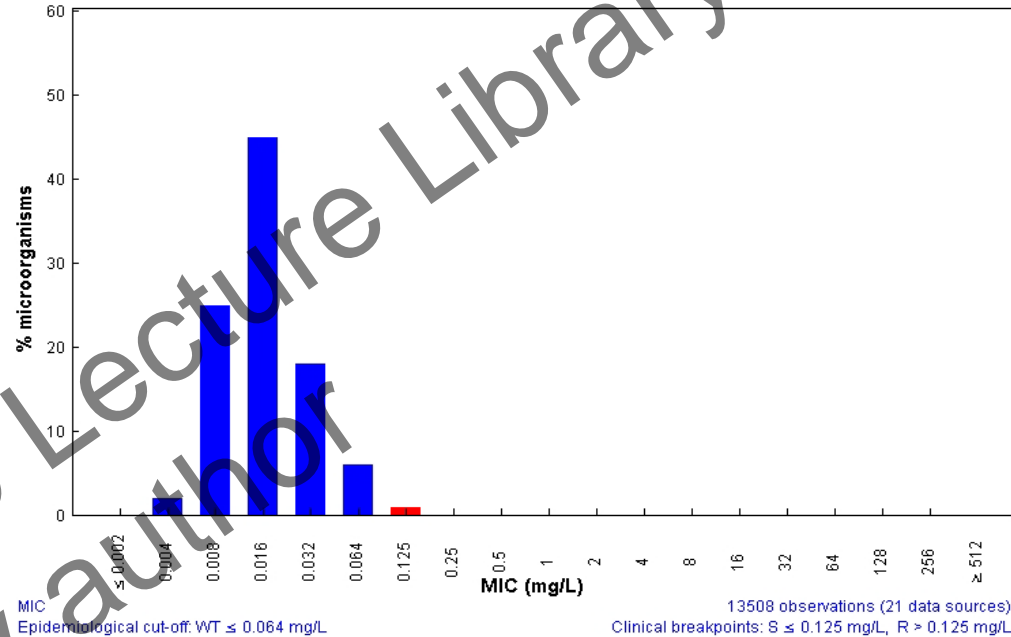
H. influenzae with cefotaxime

EUCAST database
13508 observations
21 data sources

Broth microdilution with
MH-F broth
150 isolates tested in
duplicate

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



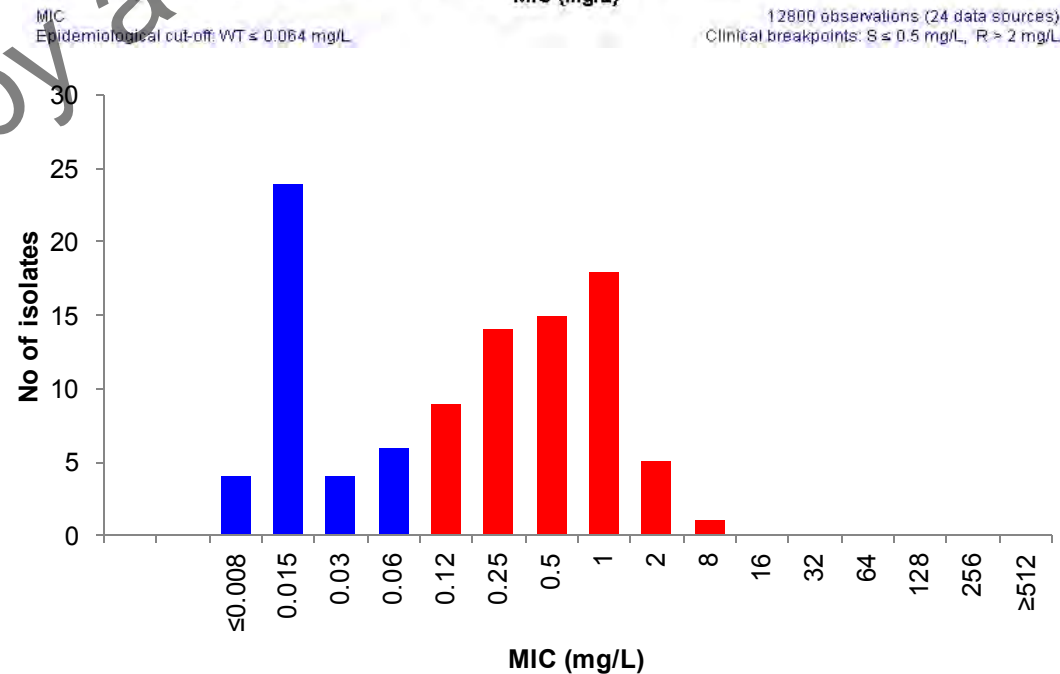
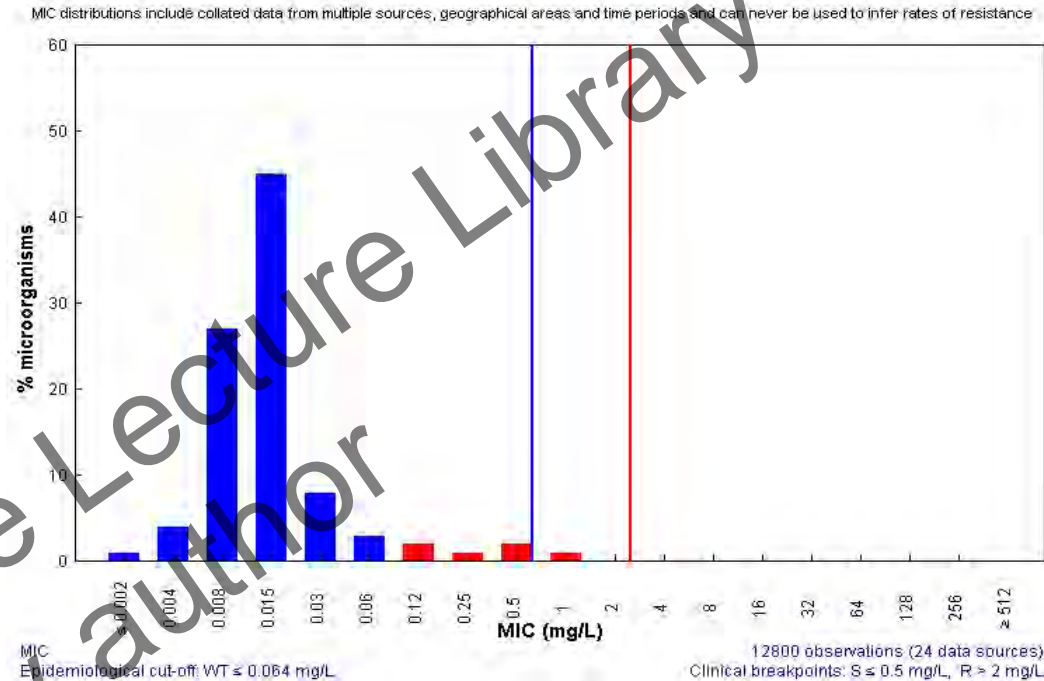
Validation of MH-F broth

S. pneumoniae with cefotaxime

EUCAST database
12800 observations
24 data sources

Broth microdilution with
MH-F broth
100 isolates

Cefotaxime / *Streptococcus pneumoniae*
EUCAST MIC Distribution - Reference Database 2013-02-21



Gradient MIC tests on MH-F medium

- Details of tests validated by manufacturer listed on EUCAST website
http://www.eucast.org/antimicrobial_susceptibility_testing/compliance_of_manufacturers

Compliance of manufacturers of AST materials and devices with EUCAST guidelines

- Data are based on information from manufacturers
- The accuracy of data in these tables is not verified by EUCAST and the inclusion of any materials or devices does not indicate endorsement by EUCAST.

Gradient tests: Etest

Manufacturer	Agents in EUCAST tables but not available	Validated for MH-F	β-lactamase inhibitor format
bioMérieux	Cefadroxil Cefalexin Cefazolin Ceftibuten Roxithromycin Ticarcillin	Agents validated for MH-F: Amoxicillin-clavulanate Ampicillin Ampicillin-sulbactam Benzylpenicillin Cefaclor Cefotaxime Ceftriaxone Cefuroxime Doripenem Ertapenem Imipenem Meropenem Chloramphenicol Clindamycin Erythromycin Levofloxacin Moxifloxacin Ofloxacin Tetracycline Trimethoprim-sulfamethoxazole (not for <i>H. influenzae</i> and <i>M. catarrhalis</i>) Vancomycin	Fixed 2:1 ratio: Amoxicillin-clavulanate Ampicillin-sulbactam Fixed inhibitor concentration (mg/L): Piperacillin-tazobactam (4) Ticarcillin-clavulanate (2)

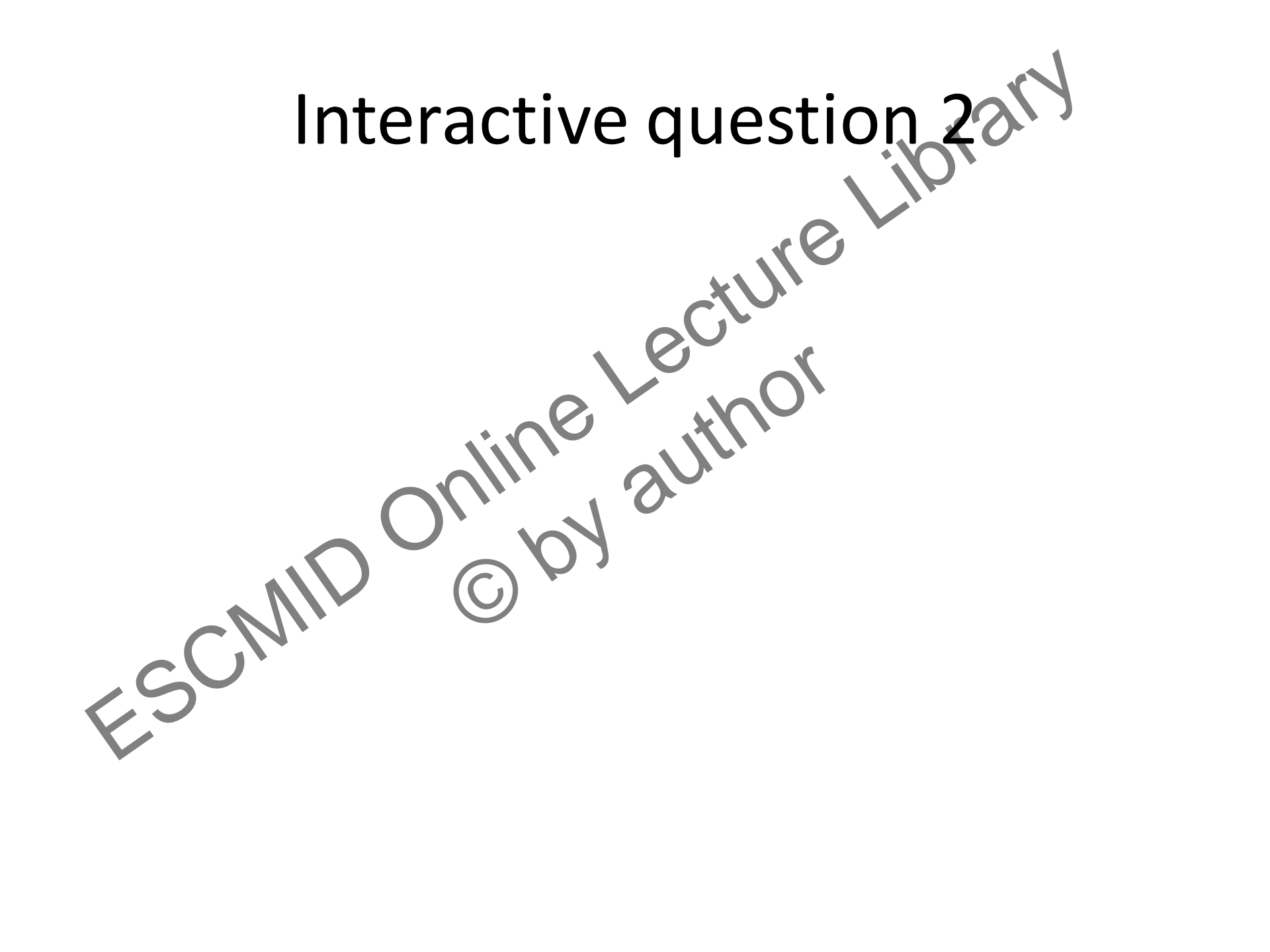
Gradient tests: MIC Test Strip

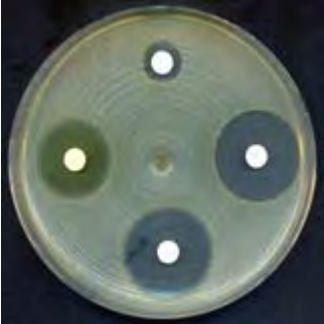
Manufacturer	Agents in EUCAST tables but not available	Validated for MH-F	β -lactamase inhibitor format
Liofilchem	Cefadroxil Cefalexin Cefazolin Ceftibuten Roxithromycin Telithromycin Ticarcillin	Amoxicillin Amoxicillin-clavulanate Ampicillin Ampicillin-sulbactam Azithromycin Benzylpenicillin Cefaclor Cefepime Cefixime Cefotaxime Cefpodoxime Ceftaroline Ceftriaxone Cefuroxime Cephalothin Chloramphenicol Ciprofloxacin Clarithromycin	<p>Fixed 2:1 ratio: Amoxicillin-clavulanate Ampicillin-sulbactam</p> <p>Fixed inhibitor concentration (mg/L): Amoxicillin-clavulanate (2) Ampicillin-sulbactam (4) Piperacillin-tazobactam (4) Ticarcillin-clavulanate (2)</p>

Gradient tests: M.I.C.Evaluator

Manufacturer	Agents in EUCAST tables but not available		Validated for MH-F	β -lactamase inhibitor format
Thermo Fisher Scientific (Oxoid)	Ampicillin-sulbactam Azithromycin Aztreonam Cefaclor Cefadroxil Cefalexin Cefazolin Cefepime Cefixime Cefoxitin Cefpodoxime Ceftributen Cefuroxime Chloramphenicol Clarithromycin Colistin Doripenem Doxycycline Ertapenem Fosfomicin Fusidic acid Mecillinam Minocycline Moxifloxacin	Mupirocin Nalidixic acid Netilmicin Nitrofurantoin Norfloxacin Ofloxacin Piperacillin Piperacillin-tazobactam Quinupristin-dalfopristin Rifampicin Roxithromycin Spectinomycin Streptomycin Telavancin Telithromycin Ticarcillin Ticarcillin-clavulanate Tobramycin Trimethoprim Trimethoprim-sulfamethoxazole	No. Expected in 2013.	Fixed 2:1 ratio: Amoxicillin-clavulanate

Interactive question 2





Disk diffusion



- Reading instructions for difficult tests added to breakpoint table
- *Campylobacter* spp. method and zone diameter breakpoints
- *Pasteurella multocida* method and zone diameter breakpoints
- Zone diameter breakpoints for new agents
- Revision of zone diameter breakpoints
- Screening tests
- Data on correlation of disk diffusion breakpoints with MIC breakpoints

Reading instructions for difficult disk diffusion tests in breakpoint tables

http://www.eucast.org/antimicrobial_susceptibility_testing/breakpoints

Stenotrophomonas maltophilia and
trimethoprim-sulfamethoxazole

Outer zone $\geq 16\text{mm}$ = S No trace of zone = R



Reading instructions for difficult disk diffusion tests in breakpoint tables

http://www.eucast.org/antimicrobial_susceptibility_testing/breakpoints

S. aureus and benzylpenicillin

Zone diam ≥ 26 mm

Fuzzy zone edge = S

Sharp zone edge = R



Reading instructions for difficult disk diffusion tests in breakpoint tables

http://www.eucast.org/antimicrobial_susceptibility_testing/breakpoints

Enterococcus. spp. and vancomycin

Zone diam ≥ 12 mm

Sharp zone edge = S

Fuzzy zone edge = R



EUCAST disk diffusion method for *Campylobacter* spp. (*jejuni* and *coli*)

Media	MH-F (pre-dried plates)
Inoculum	McFarland 0.5
Incubation	Microaerobic environment 41 C 24 h*
Reading	EUCAST standard reading

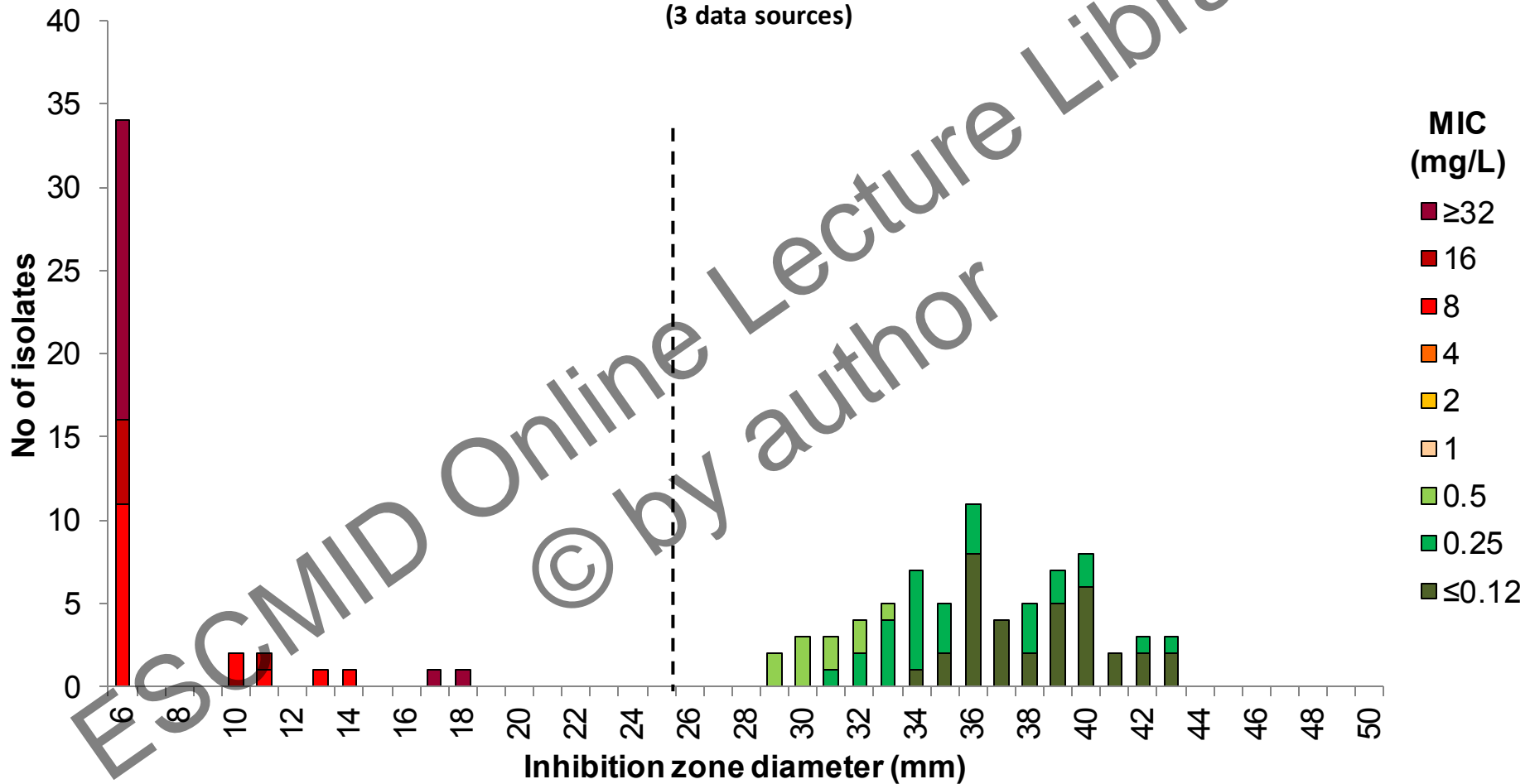
* *Campylobacter coli* isolates with insufficient growth are reincubated immediately and read after a total of 40-48 h incubation

Calibration of disk diffusion method for *Campylobacter* spp.

http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation

- 30 *C. jejuni* and 27 *C. coli* from two collections
- MIC by ISO broth microdilution
- Disk diffusion in duplicate by EUCAST method
- Study in collaboration between EUCAST, Central Veterinary Institute (CVI), Lelystad, the Netherlands and National Institute for Health and Welfare (THL), Turku, Finland.

Ciprofloxacin 5 µg vs. MIC, *Campylobacter jejuni* and *coli* 57 isolates tested in duplicate



Breakpoints

MIC

S ≤ 0.5, R > 0.5 mg/L

Zone diameter

S ≥ 26, R < 26 mm

ECOFF

WT ≤ 0.5 mg/L

EUCAST disk diffusion method for *Pasteurella multocida*

Medium	MH-F
Inoculum	McFarland 0.5
Incubation	5% CO ₂ , 35 °C, 18 h 2 h
Reading	Read zone edges at the point of complete inhibition of growth viewed from the front of the plate with the lid removed and with reflected light

Calibration of disk diffusion method for *Pasteurella multocida*

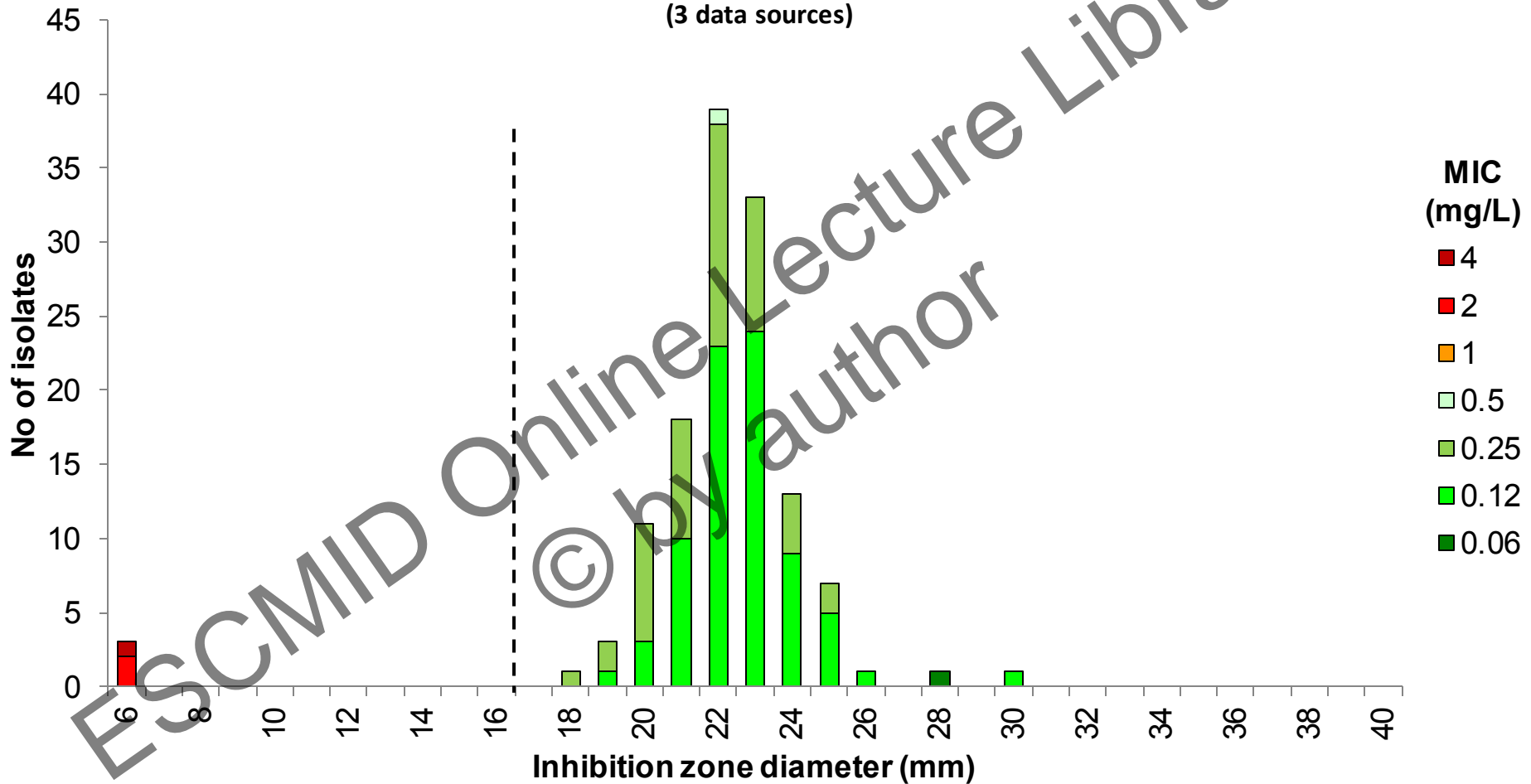
http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation

- 131 *P. multocida* from two collections
- MIC by gradient tests on MH-F agar
- Disk diffusion in duplicate by EUCAST method
- Study in collaboration between EUCAST, Central Veterinary Institute (CVI), Lelystad, the Netherlands and JMI Laboratories, North Liberty, Iowa, USA

Benzylpenicillin 1 unit vs. MIC

Pasteurella multocida, 131 isolates

(3 data sources)



Breakpoints

MIC $S \leq 0.5, R > 0.5$ mg/L

Zone diameter $S \geq 17, R < 17$ mm

ECOFF

WT ≤ 0.5 mg/L

Zone diameter breakpoints for new agents

- MIC breakpoints set as part of the marketing authorisation through EMA
- 2012 Ceftaroline

Organisms	MIC breakpoints (mg/L)		Zone diameter breakpoints (mm)	
	S ≤	R >	S ≥	R <
<i>Staphylococcus aureus</i>	1	1	20	20
<i>Streptococcus pneumoniae</i>	0.25	0.25	Note ²	Note ²
Streptococcus Groups A, B, C, G	Note ¹	Note ¹	Note ¹	Note ¹
<i>Haemophilus influenzae</i>	0.03	0.03	IP	IP
Enterobacteriaceae	0.5	0.5	23	23

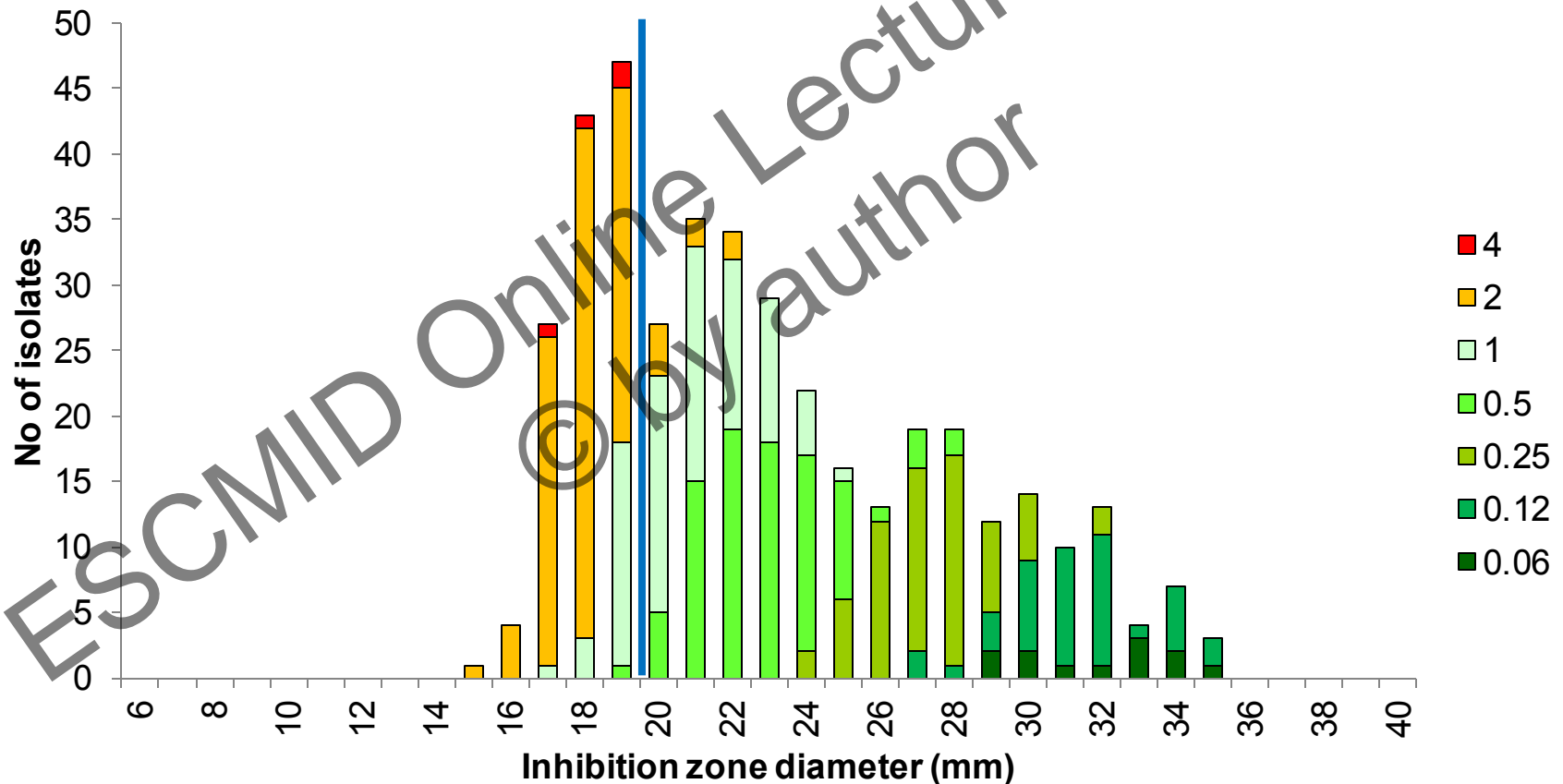
1. Infer susceptibility from susceptibility to benzylpenicillin

2. Screen with oxacillin disk

IP = In preparation

Zone diameter breakpoints for new agents - ceftaroline

Ceftaroline 5 µg vs. MIC
S. aureus, 100 clinical isolates tested at 2 sites x2



Note in breakpoint table: For isolates with zone diameters 19-21 mm, determine the MIC to confirm the susceptibility

Revision of zone diameter breakpoints

- Breakpoints may be revised in the light of new information and changes highlighted in breakpoint tables
(http://www.eucast.org/clinical_breakpoints)
- e.g. Telithromycin and strep groups A,B,C,G
 - 2012 S \geq 22 mm, R <19 mm
 - 2013 S \geq 20 mm, R <17 mm
- e.g. Teicoplanin and *S. pneumoniae*
 - 2012 S \geq 18 mm, R <18 mm
 - 2013 S \geq 17 mm, R <17 mm

Screening for beta-lactam resistance in *S. pneumoniae*

Classical oxacillin screen for beta-lactam non-susceptibility in *S. pneumoniae*

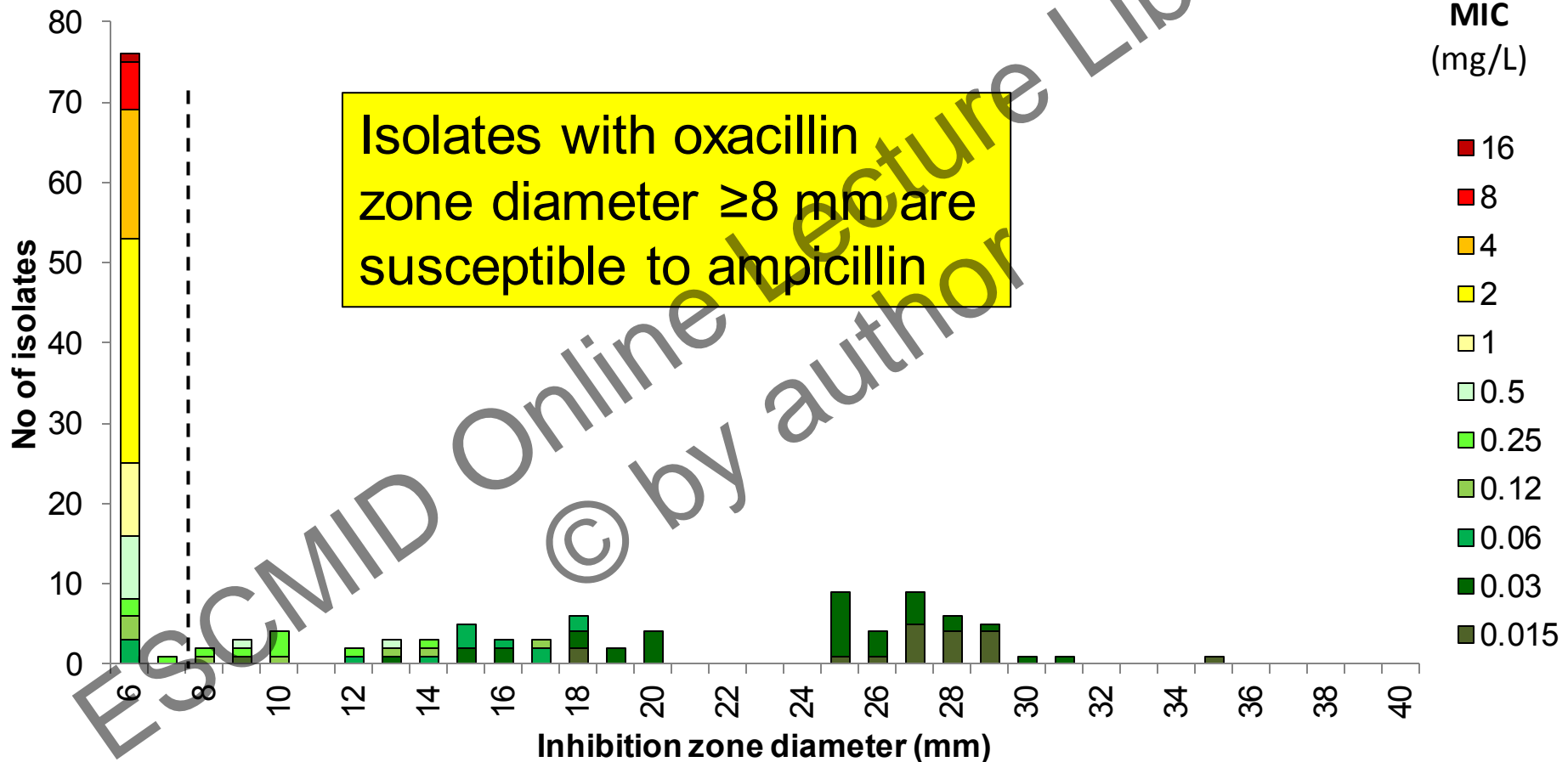
$S \geq 20$, $R < 20$ mm

Oxacillin screen can give useful information on susceptibility to beta-lactam agents other than benzylpenicillin

Detailed data on EUCAST website

http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation

Oxacillin 1 µg vs. ampicillin MIC *S. pneumoniae*, 153 clinical isolates



Breakpoints

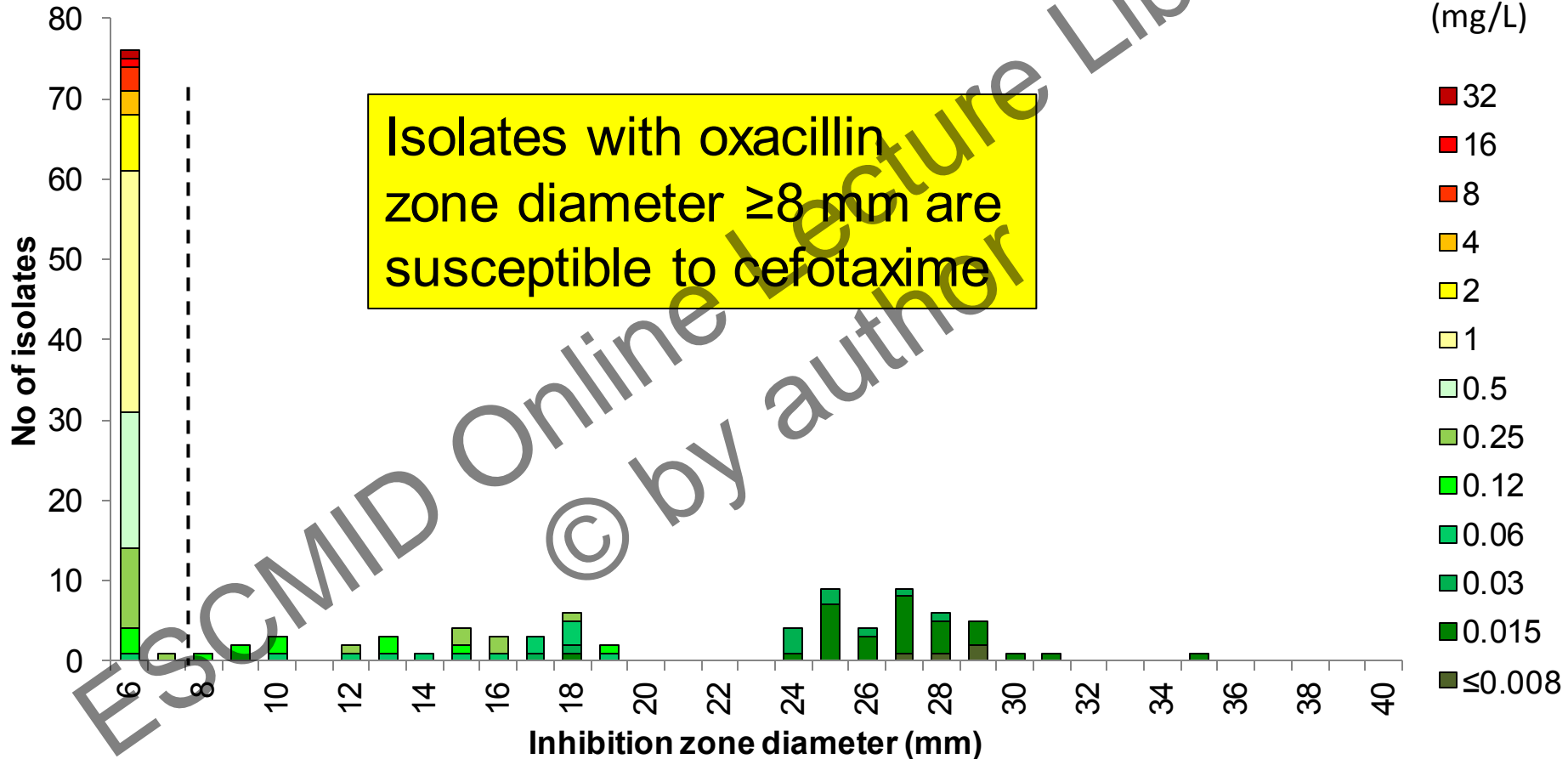
Ampicillin MIC

$S \leq 0.5$, $R > 2$ mg/L

Oxacillin zone diameter (screen)

Ampicillin $S \geq 8$ mm

Oxacillin 1 μ g vs. cefotaxime MIC *S. pneumoniae*, 147 clinical isolates



Breakpoints

Cefotaxime MIC

$S \leq 0.5$, $R > 2$ mg/L

Oxacillin zone diameter (screen)

Cefotaxime $S \geq 8$ mm

Screening for beta-lactam resistance in *S. pneumoniae*

	Oxacillin zone diameter ≥ 8 mm: Report susceptible.
Ampicillin, amoxicillin and piperacillin (without and with beta-lactamase inhibitor), cefepime, cefotaxime and ceftriaxone	Oxacillin zone diameter < 8 mm: determine the MIC of the beta-lactam agent intended for clinical use but for ampicillin, amoxicillin and piperacillin (without and with beta-lactamase inhibitor) infer susceptibility from the MIC of ampicillin.
Other beta-lactam agents	Test by an MIC method for the agent considered for clinical use and interpret according to the clinical breakpoints.

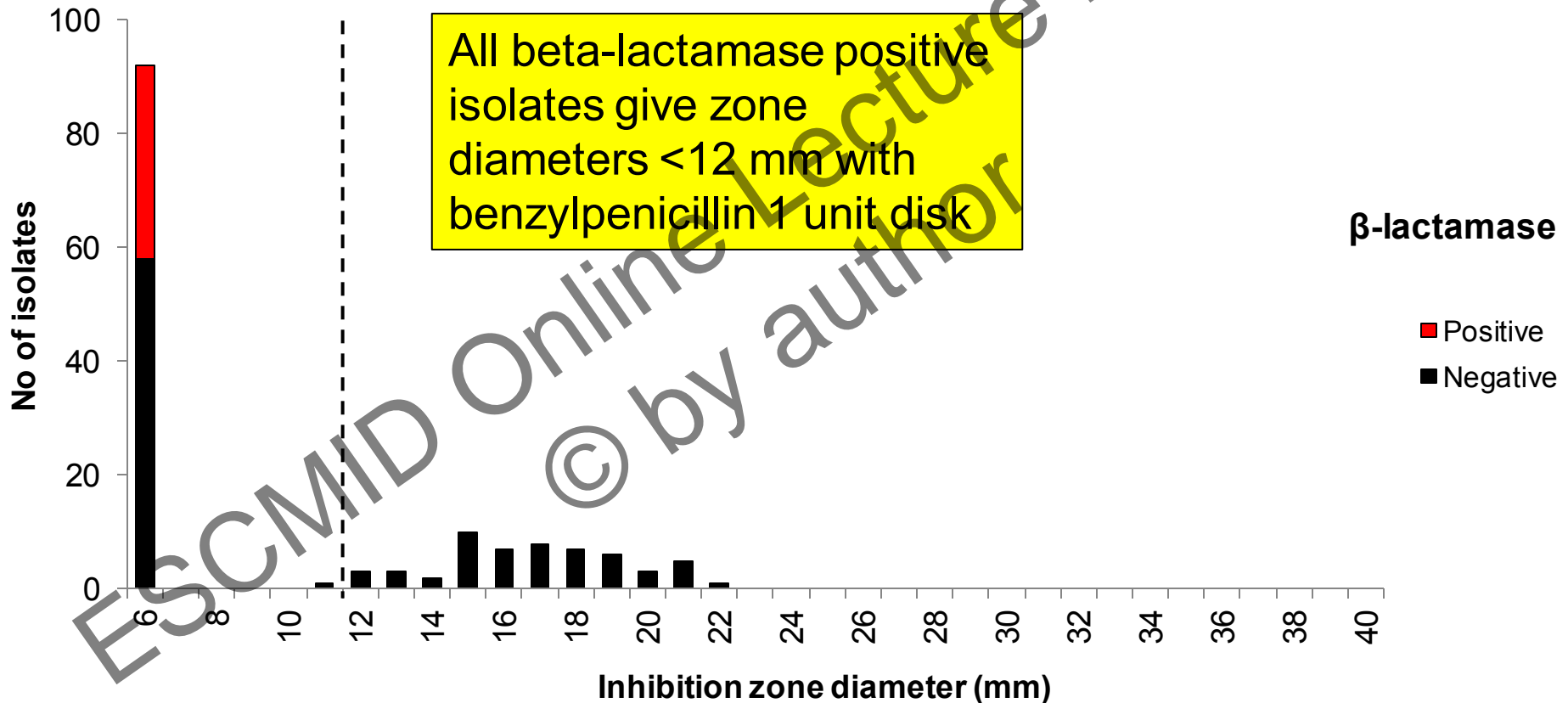
Screening for beta-lactam resistance in *H. influenzae*

Benzyloenicillin screen can give useful information on susceptibility to beta-lactam agents

Detailed data on EUCAST website

http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation

Benzylpenicillin 1 unit vs. *H. influenzae* with or without β -lactamase, 148 clinical isolates

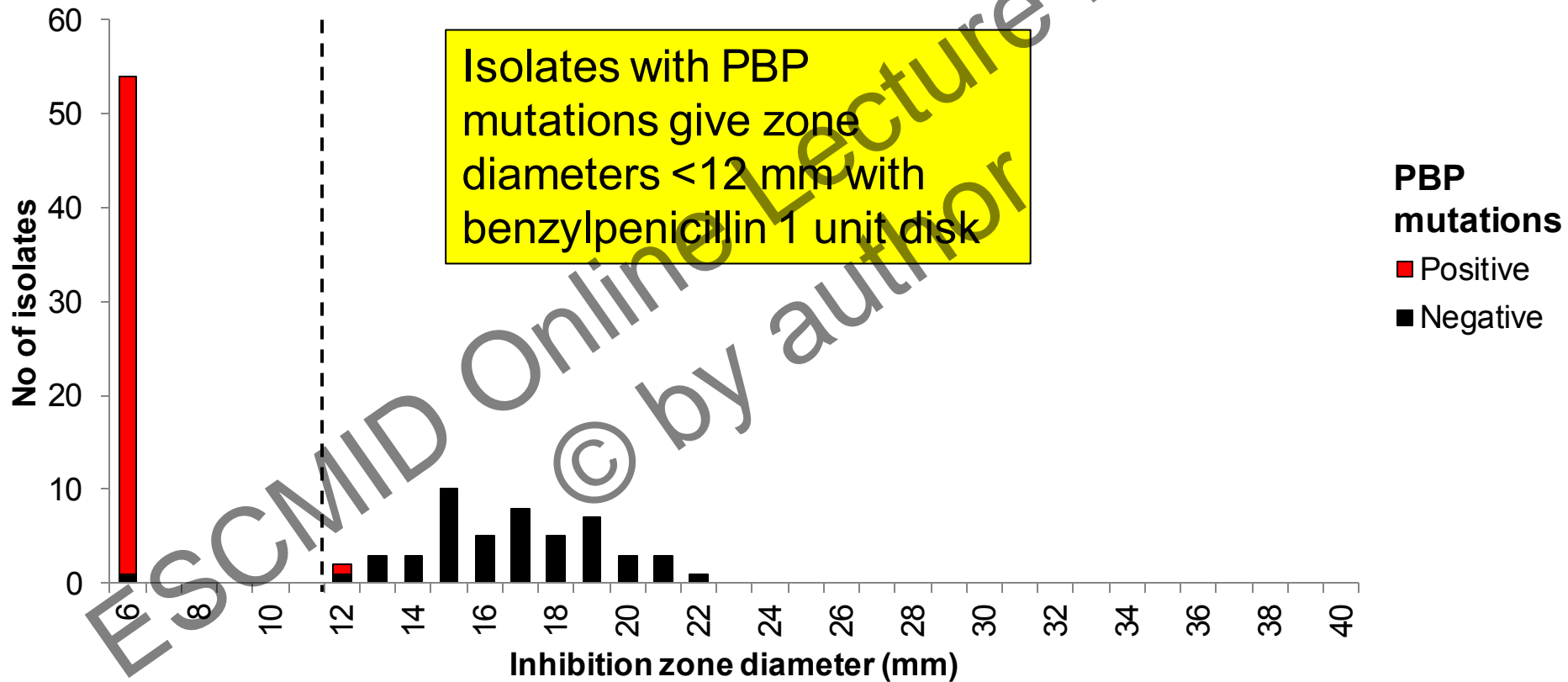


Breakpoints

Benzylpenicillin zone diameter (screen)

S \geq 12, R<12 mm

Benzylpenicillin 1 unit vs. *H. influenzae* with or without PBP mutations, 104 β -lactamase-negative clinical isolates



Breakpoints
Benzylpenicillin zone diameter (screen) $S \geq 12$, $R < 12$ mm

Screening for beta-lactam resistance in *H. influenzae*

Benzylpenicillin 1 unit disk, Zone diameter (mm)	Beta-lactamase	Further testing and/or interpretation
≥ 12 mm	Do not test	Report susceptible to all beta-lactam agents for which clinical breakpoints are listed (including those with “Note”).
< 12 mm	Beta-lactamase negative	Test susceptibility to the beta-lactam agent intended for clinical use.
	Beta-lactamase positive	For ampicillin, amoxicillin and piperacillin, report resistant. For other beta-lactam agents, test susceptibility to the beta-lactam agent intended for clinical use.

Screening for beta-lactam resistance in *H. influenzae*

Benzylpenicillin 1 unit disk, Zone diameter (mm)	Beta-lactamase	Further testing and/or interpretation
≥ 12 mm	Do not test	Report susceptible to all beta-lactam agents for which clinical breakpoints are listed (including those with “Note”).
< 12 mm	Beta-lactamase negative	Test susceptibility to the beta-lactam agent intended for clinical use.
	Beta-lactamase positive	For ampicillin, amoxicillin and piperacillin, report resistant. For other beta-lactam agents, test susceptibility to the beta-lactam agent intended for clinical use.

Screening for beta-lactam resistance in *H. influenzae*

Benzylpenicillin 1 unit disk, Zone diameter (mm)	Beta-lactamase	Further testing and/or interpretation
≥ 12 mm	Do not test	Report susceptible to all beta-lactam agents for which clinical breakpoints are listed (including those with "Note").
< 12 mm	Beta-lactamase negative	Test susceptibility to the beta-lactam agent intended for clinical use.
	Beta-lactamase positive	For ampicillin, amoxicillin and piperacillin, report resistant. For other beta-lactam agents, test susceptibility to the beta-lactam agent intended for clinical use.

Screening for beta-lactam resistance in *H. influenzae*

Benzylpenicillin 1 unit disk, Zone diameter (mm)	Beta-lactamase	Further testing and/or interpretation
≥ 12 mm	Do not test	Report susceptible to all beta-lactam agents for which clinical breakpoints are listed (including those with “Note”).
< 12 mm	Beta-lactamase negative	Test susceptibility to the beta-lactam agent intended for clinical use.
	Beta-lactamase positive	For ampicillin, amoxicillin and piperacillin, report resistant. For other beta-lactam agents, test susceptibility to the beta-lactam agent intended for clinical use.

Data on correlation of zone diameter breakpoints with EUCAST MIC breakpoints

Extensive new data added to EUCAST website

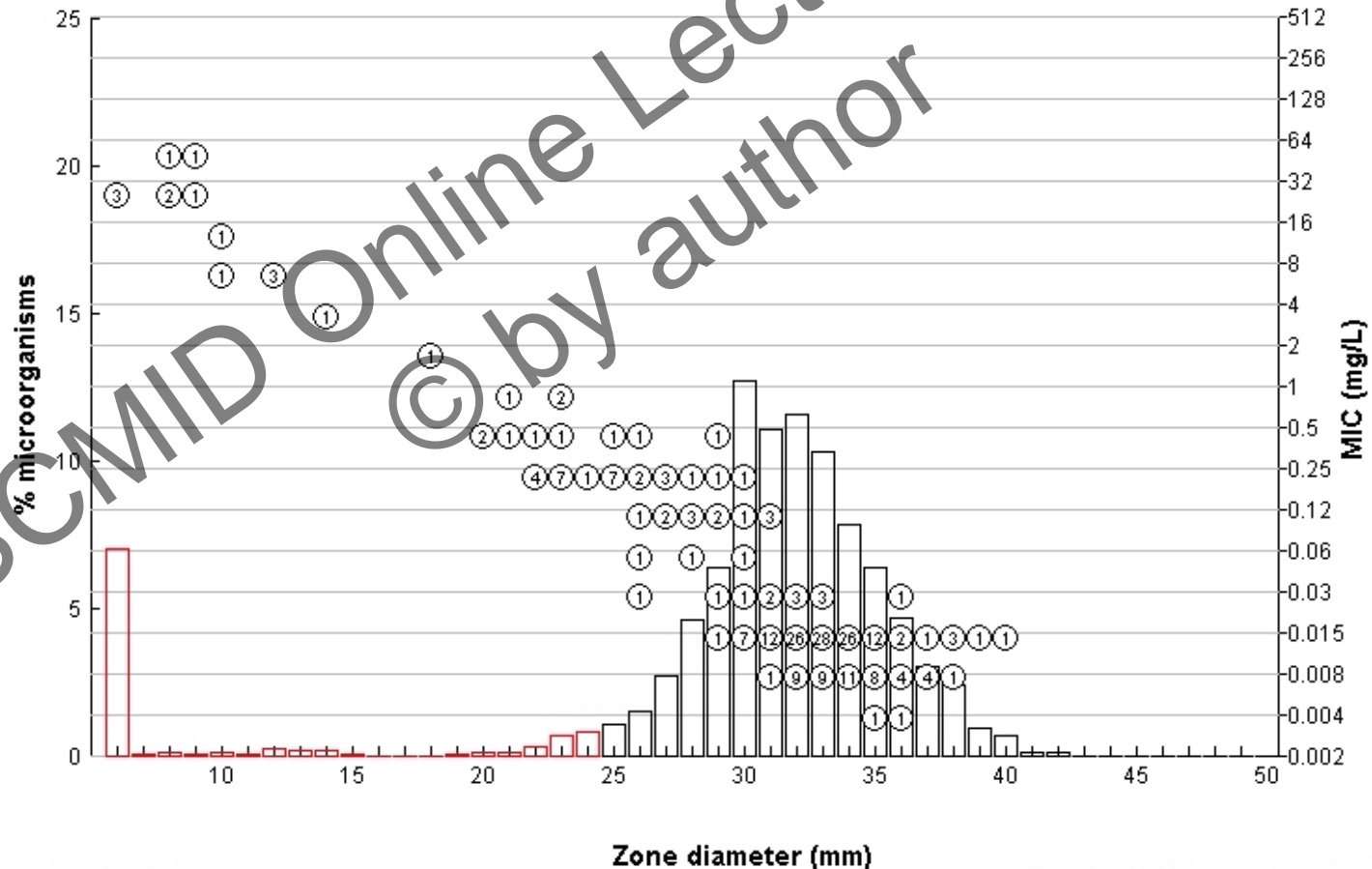
- MIC-zone diameter distributions
- Zone diameter distributions with MIC data identified in histograms

MIC-zone diameter distributions

<http://mic.eucast.org/Eucast2>

Ciprofloxacin / Escherichia coli EUCAST zone diameter distribution - Reference database 2012-11-29 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: 5

Epidemiological cut-off: WT \geq 25 mm (MIC \leq 0.064 mg/L)

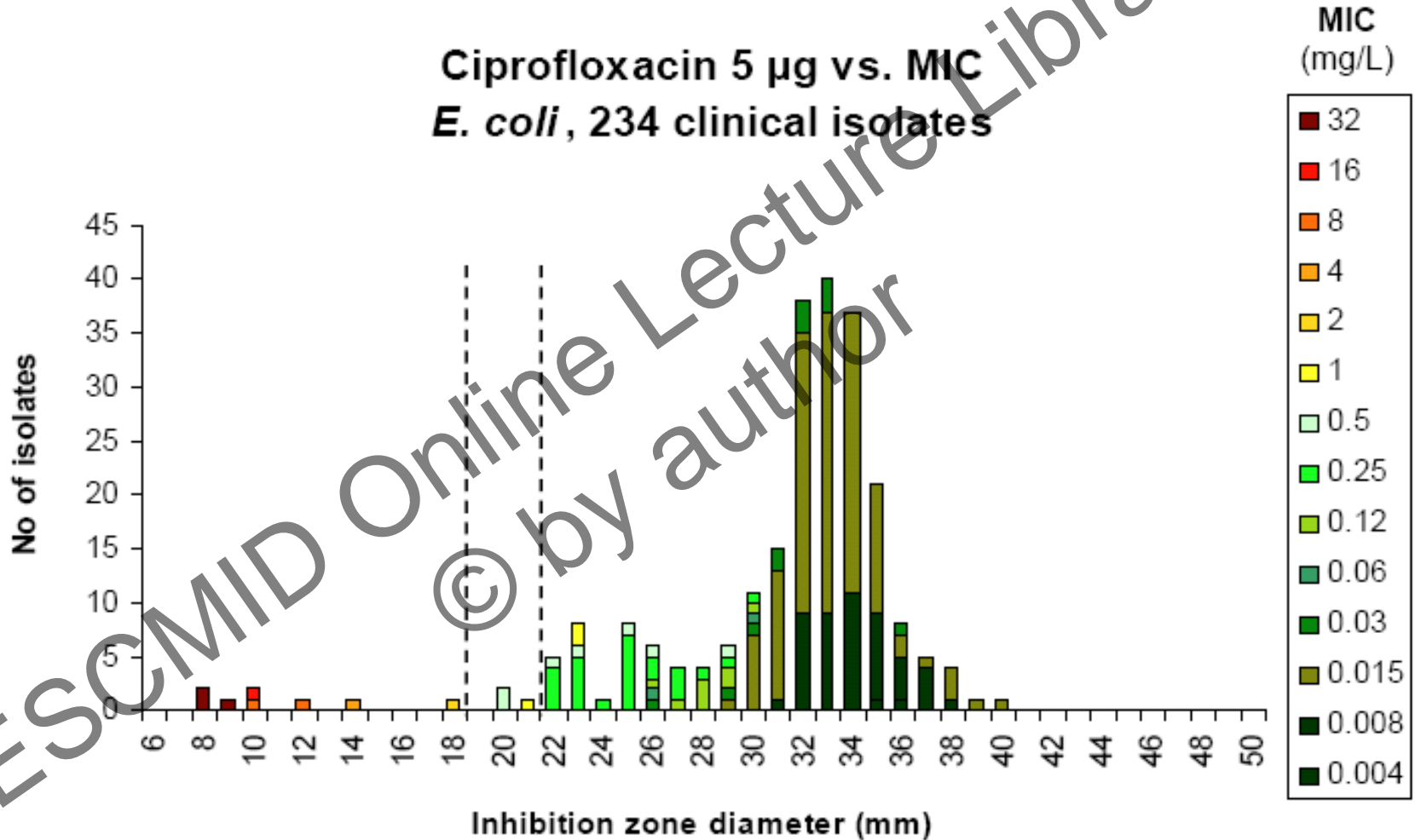
6434 observations (6 data sources)

Clinical breakpoints: S \geq 22 mm, R < 19 mm (S \leq 0.5 mg/L, R > 1 mg/L,)

Zone diameter distributions with MICs

http://www.eucast.org/antimicrobial_susceptibility_testing/calibration_and_validation

Ciprofloxacin 5 µg vs. MIC
***E. coli*, 234 clinical isolates**



Breakpoints

MIC S ≤ 0.5, R > 1 mg/L

Zone diameter S ≥ 22, R < 19 mm

ECOFF

WT ≤ 0.06 mg/L

EUCAST methods

What is coming in 2013-14?

- MIC
 - Further validation of gradient tests
- Disk diffusion
 - New agents authorised by EMA
 - *Corynebacterium* spp.
 - *Pseudomonas non-aeruginosa*
 - *Neisseria gonorrhoeae*
 - Some rapidly-growing anaerobes
- Guidance note on *Burkholderia cepacia*

[Organization](#)

[EUCAST News](#)

[Clinical breakpoints](#)

[Expert rules](#)

[Setting breakpoints](#)

[MIC distributions](#)

[Zone diameter distributions](#)

Antimicrobial susceptibility testing

[Media preparation](#)

[MIC determination](#)

[Disk diffusion methodology](#)

[Disk diffusion implementation](#)

[Compliance of manufacturers](#)

[Breakpoint tables](#)

[QC Tables](#)

[Calibration and validation](#)

[Guidance documents](#)

[Projects and data submission](#)

[Previous versions of tables](#)

[Antifungal susceptibility testing \(AFST\)](#)

[Frequently Asked Questions \(FAQ\)](#)

[Meetings](#)

[EUCAST Presentations](#)

[Documents](#)

[Information for industry](#)



search term [Search](#)

Antimicrobial susceptibility testing ▾

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 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

- **Guidance documents**
Guidance notes on specific susceptibility testing issues