

EUCAST

fastidious micro-organisms

G Kahlmeter

Update ECCMID 2012

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Q1

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MH-F

Q2

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Non-fastidious organisms

- EUCAST and CLSI disk diffusion recommendations are identical except for a few disk strengths.
- For all identical drug/bug combinations the internal QC strain recommendations are so far the same.
- In tables from EUCAST it is clearly marked when recommendations on QC differ between the two committees.

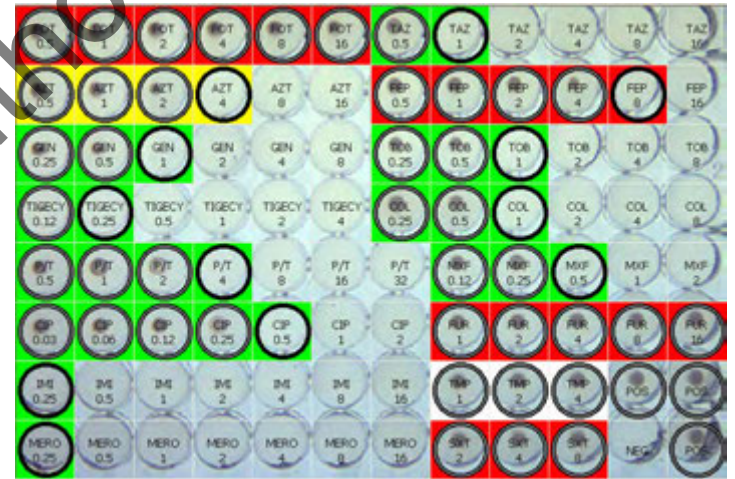
EUCAST AST of fastidious organisms on MH-F

MH-F is Mueller-Hinton agar (or broth) with 5% (mechanically) defibrinated horse blood and 20 mg/L β -NAD.

During the development we have used MH-media from 3 manufacturers with several lost from each.

More than 150 batches of plates have been manufactured in-house with few problems or variations.

Ready-made plates from several manufacturers have been compared with in-house plates with only minor variations.



EUCAST AST of fastidious organisms on MH-F

MH-F is Mueller-Hinton agar (or broth) with 5% (mechanically) defibrinated horse blood and 20 mg/L β -NAD.

Developed for:

Haemophilus influenzae

Moraxella catarrhalis

Streptococci A, B, C, G and viridans group

Streptococcus pneumoniae

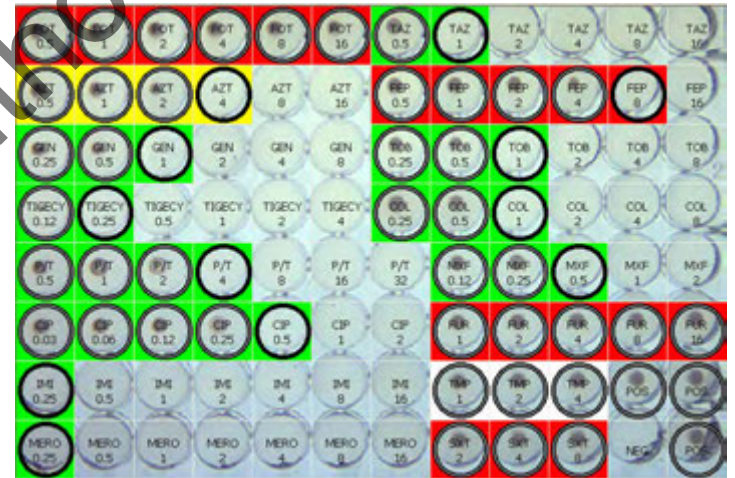
Campylobacter jejuni and *C. coli*

Listeria monocytogenes

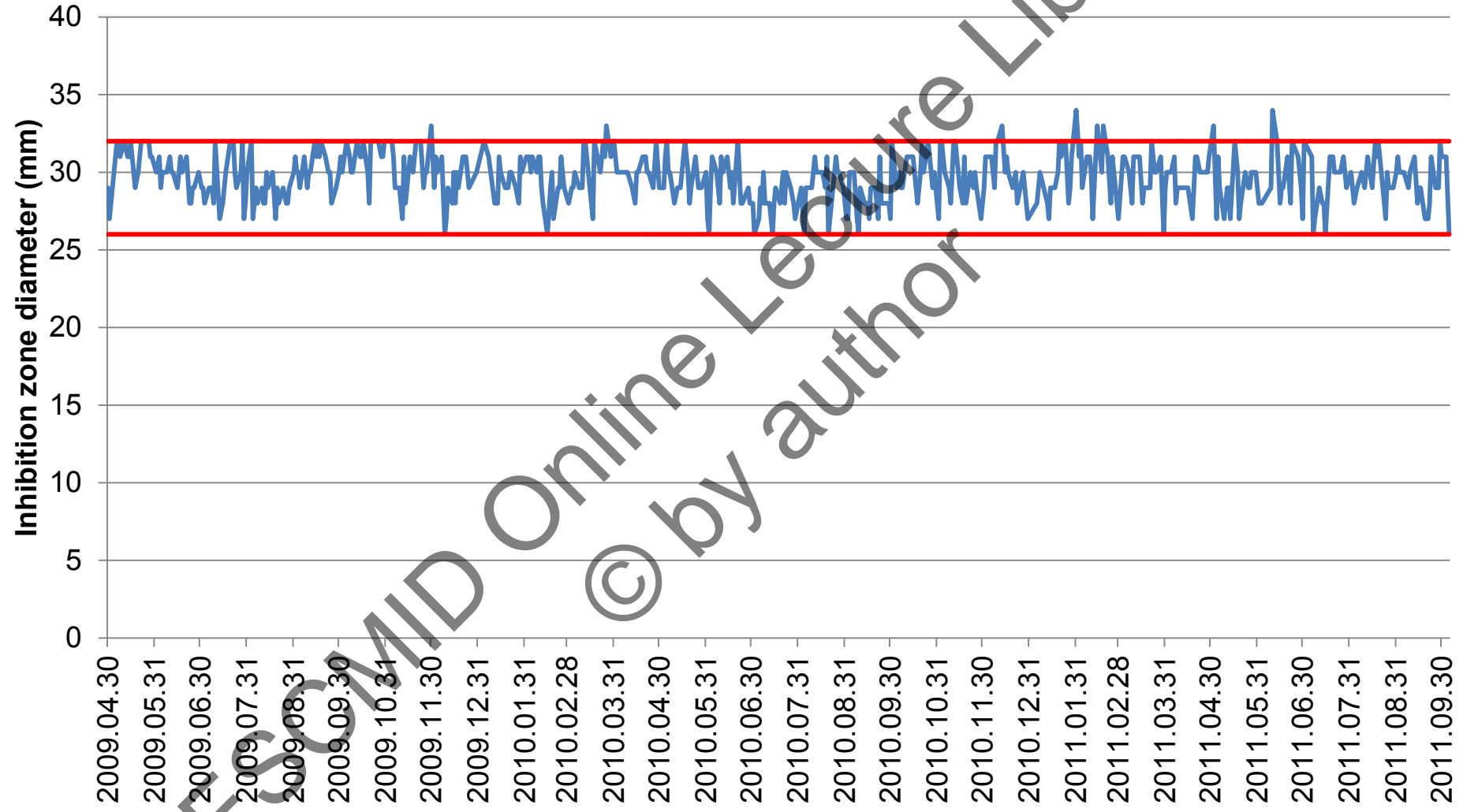
Corynebacterium spp.

Pasteurella multocida

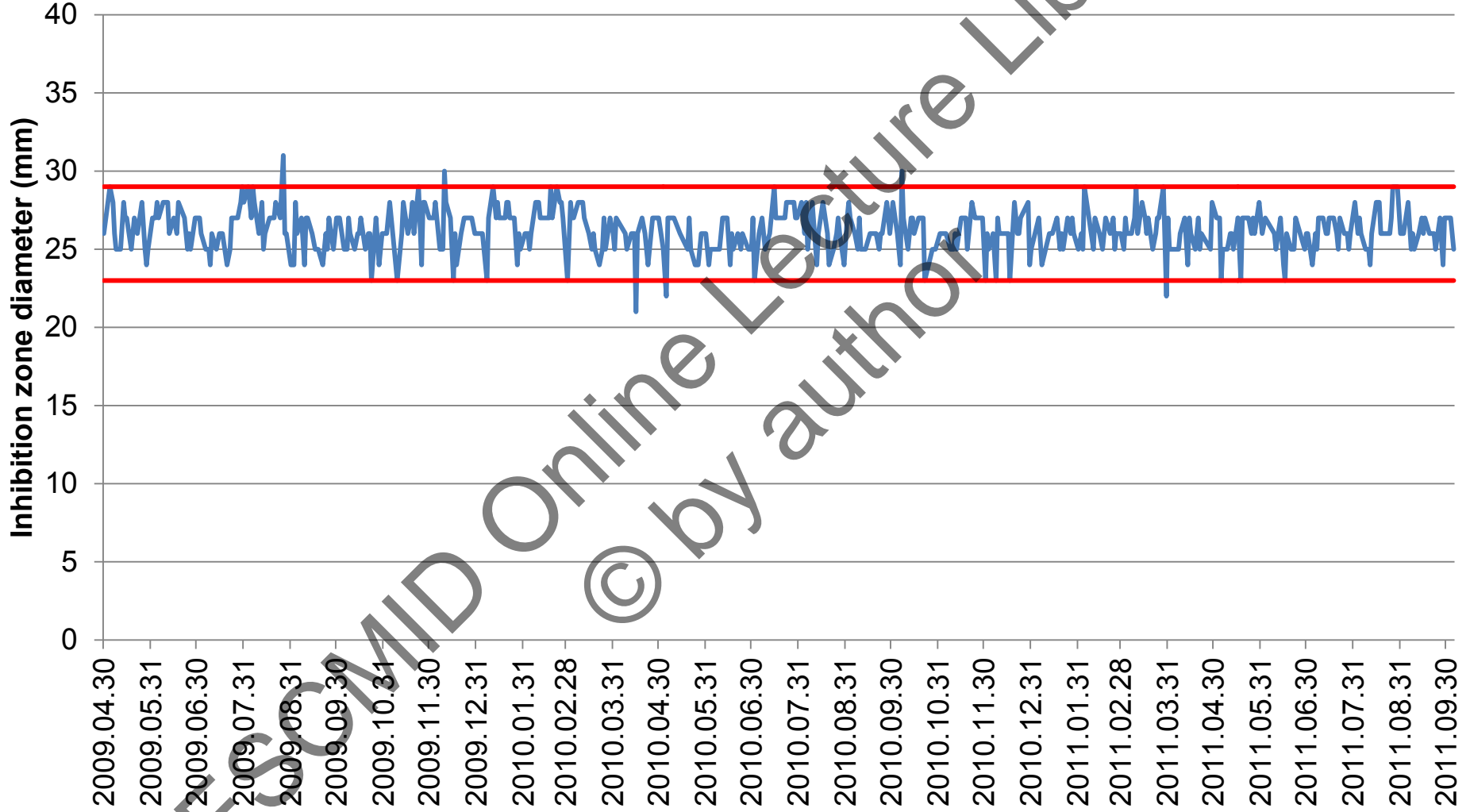
.....



***S. pneumoniae* ATCC 49619 with erythromycin 15 µg**



***S. aureus* ATCC 29213 with erythromycin 15 µg**





EUCAST

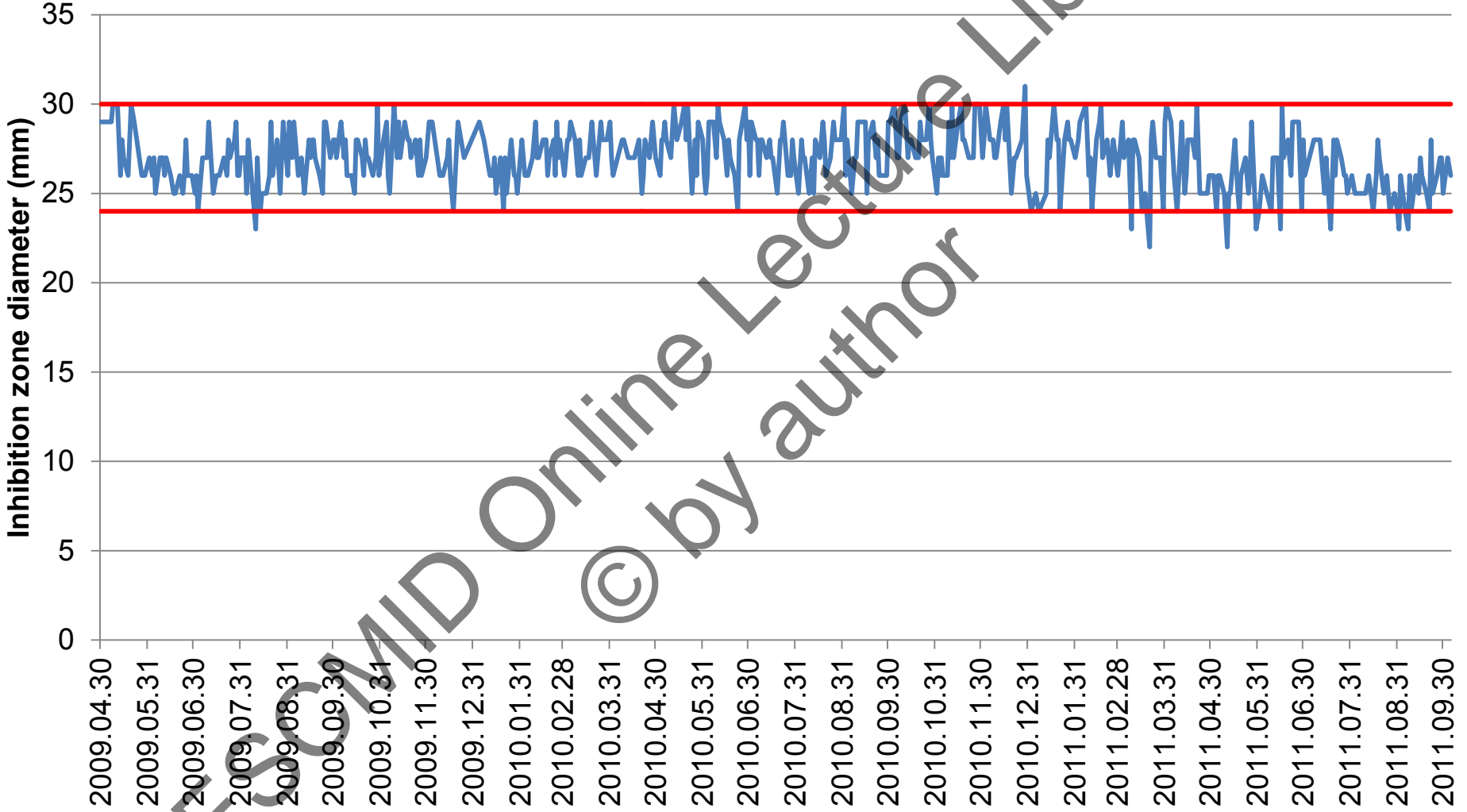
EUROPEAN COMMITTEE
ON ANTIMICROBIAL
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Haemophilus influenzae

- Susceptibility testing on
 - MH-F
 - inoculum McF 0.5
 - 5% CO₂
 - incubation 16 – 20h
- Disk diffusion breakpoints for all relevant agents.
- Screen method for betalactam resistance (β -lactamase, PBP-mediated resistance).

***H. influenzae* NCTC 8468 with cefaclor 30 µg**



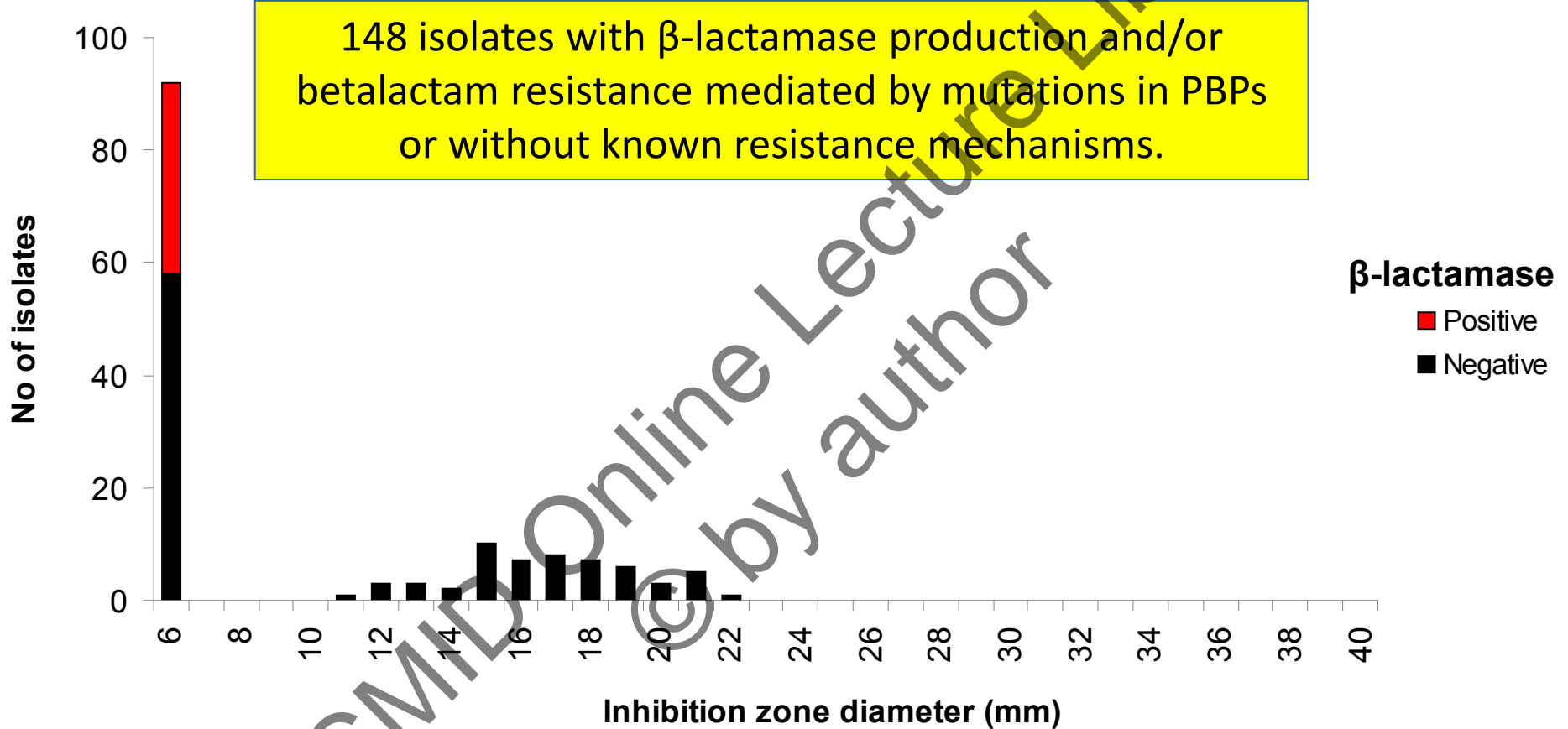
H. influenzae

– algorithm for betalactam resistance

- Test all isolates with the **Benzylpenicillin 1 unit disk**
- Isolates **≥ 12 mm** can be reported susceptible to penicillins and cephalosporins categorised as therapeutic alternatives for *H. influenzae*
- Isolates **< 12 mm** are suspected of
 - β -lactamase (15 – 30 %): ampicillin, amoxicillin
 - AND/OR**
 - PBP mutations (2 – 10 %): all betalactam antibiotics at risk, including aminopenicillins with inhibitors, cephalosporins and carbapenems. Determine susceptibility to relevant agent.

Benzylpenicillin 1 unit vs. β -lactamase *H. influenzae*, 148 clinical isolates

148 isolates with β -lactamase production and/or betalactam resistance mediated by mutations in PBPs or without known resistance mechanisms.

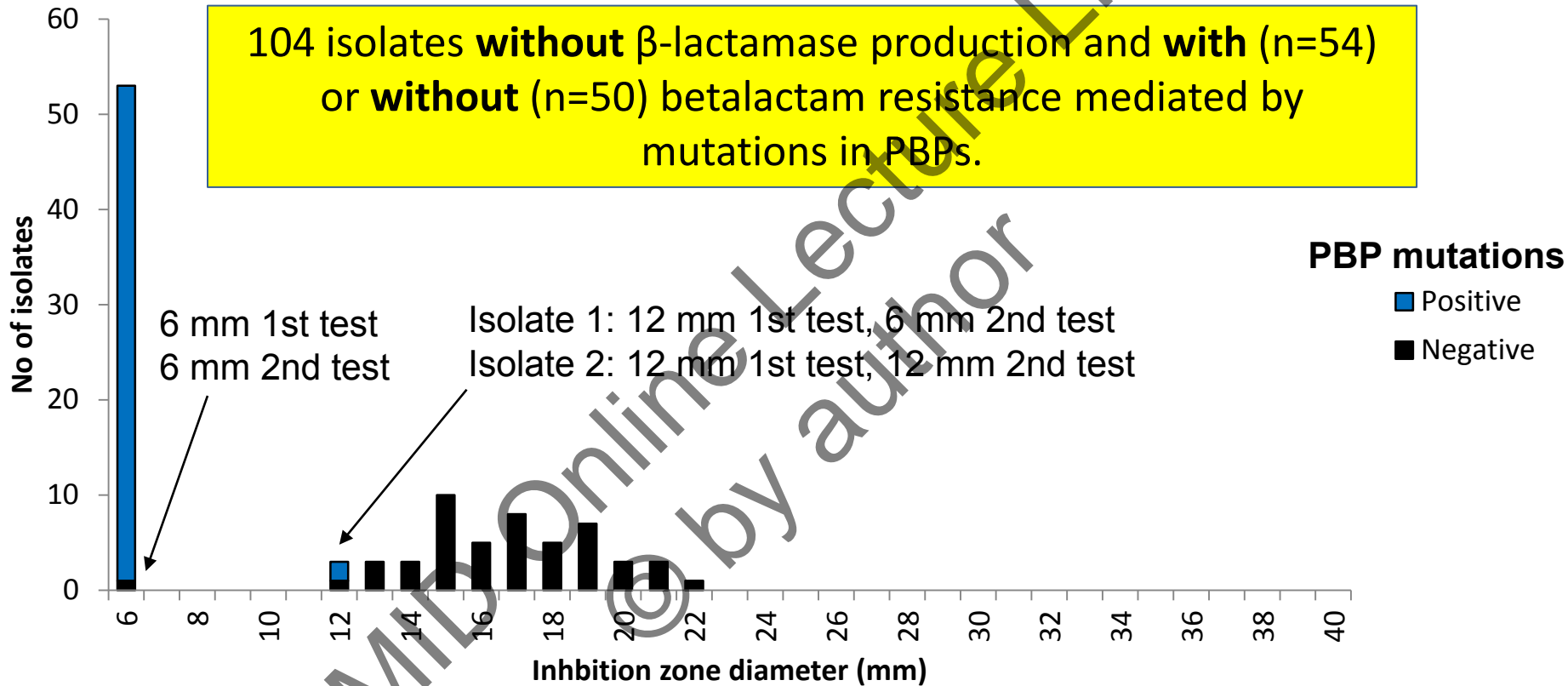


Breakpoints

Benzylpenicillin zone diameter (screen)

S \geq 12, R<12 mm

Benzylpenicillin 1 unit vs. PBP mutations *H. influenzae*, 104 β -lactamase negative clinical isolates

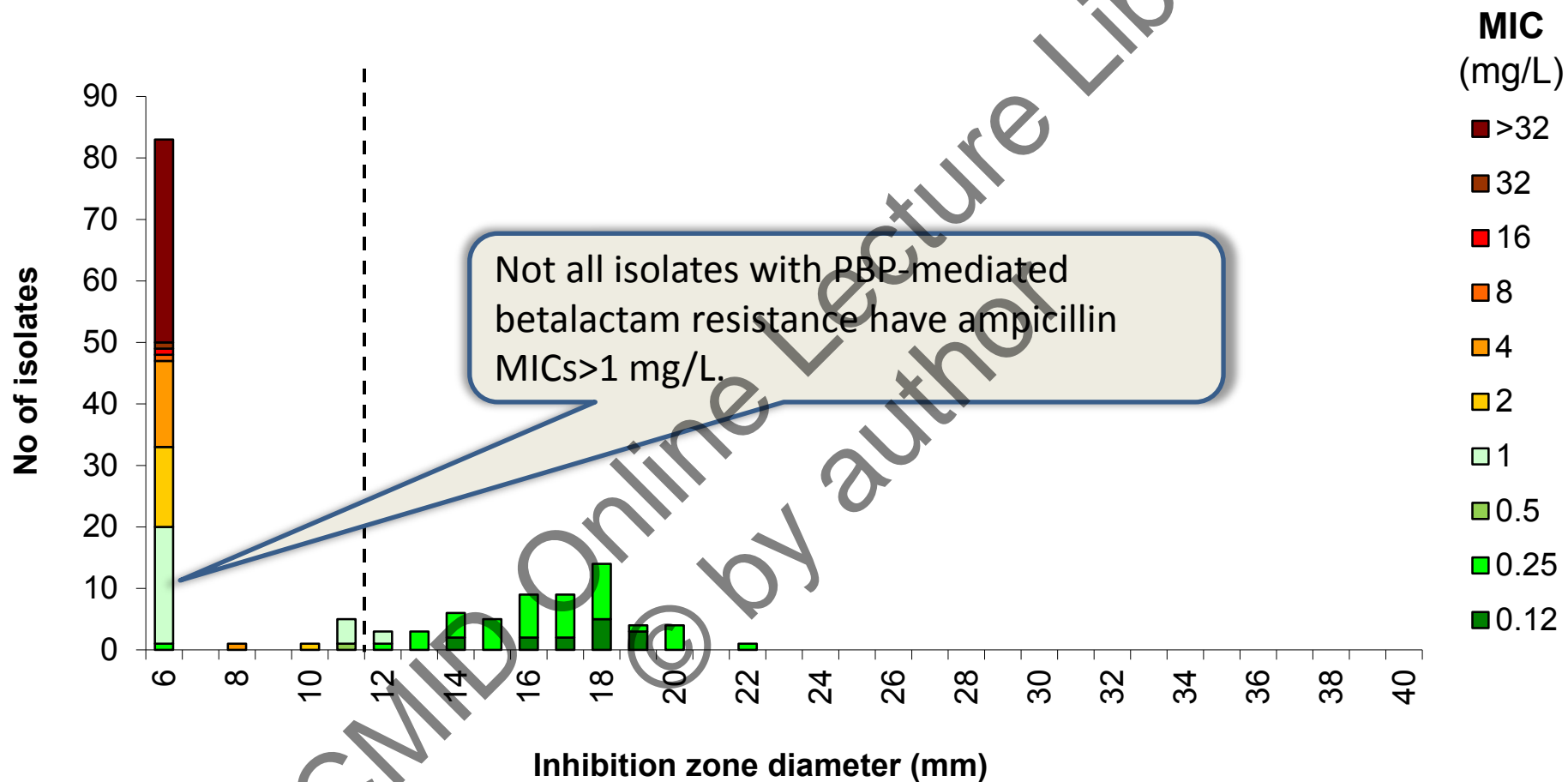


Breakpoints

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)

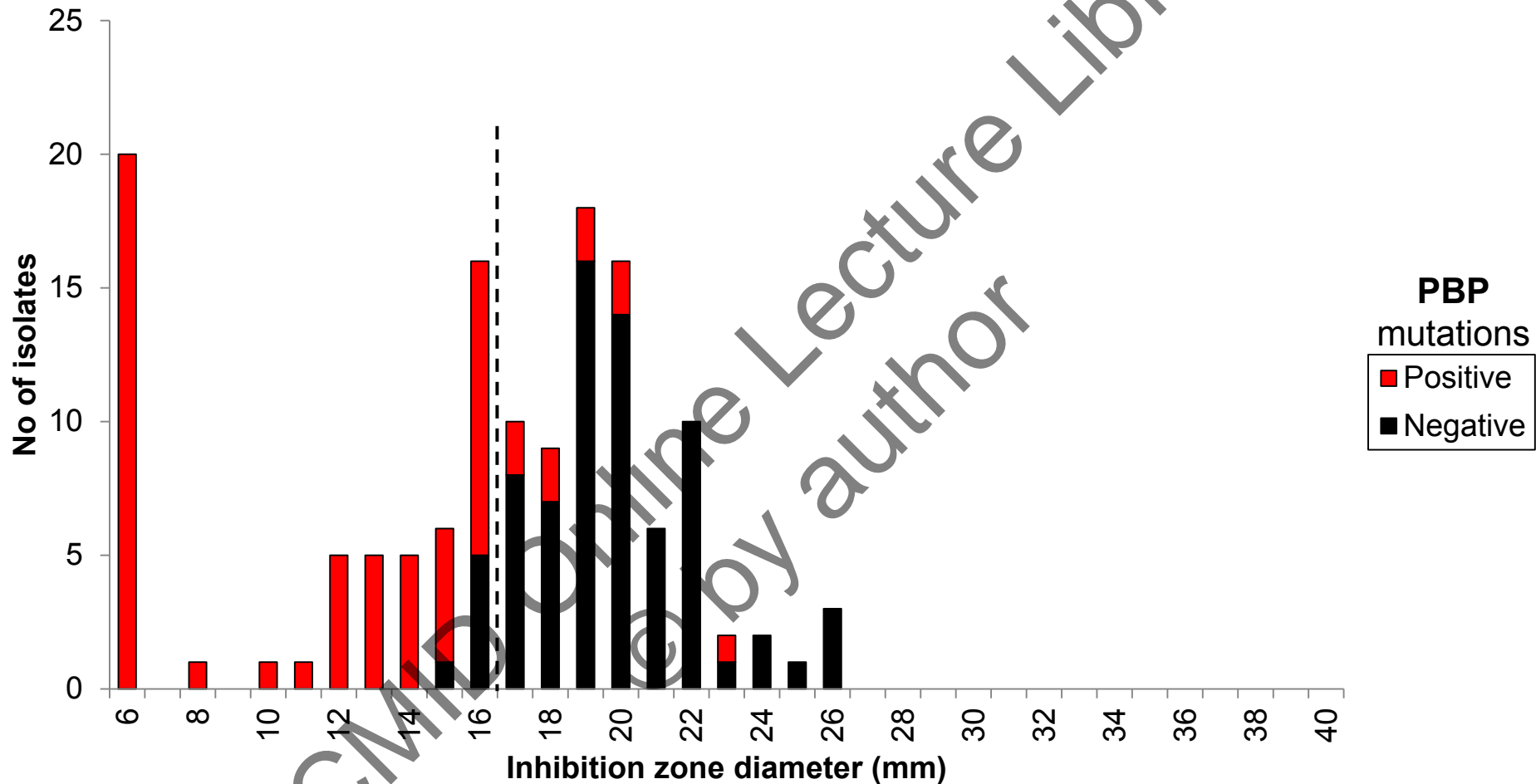
S ≤ 1, R > 1 mg/L

S ≥ 12, R < 12 mm

ECOFF

WT ≤ 1 mg/L

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



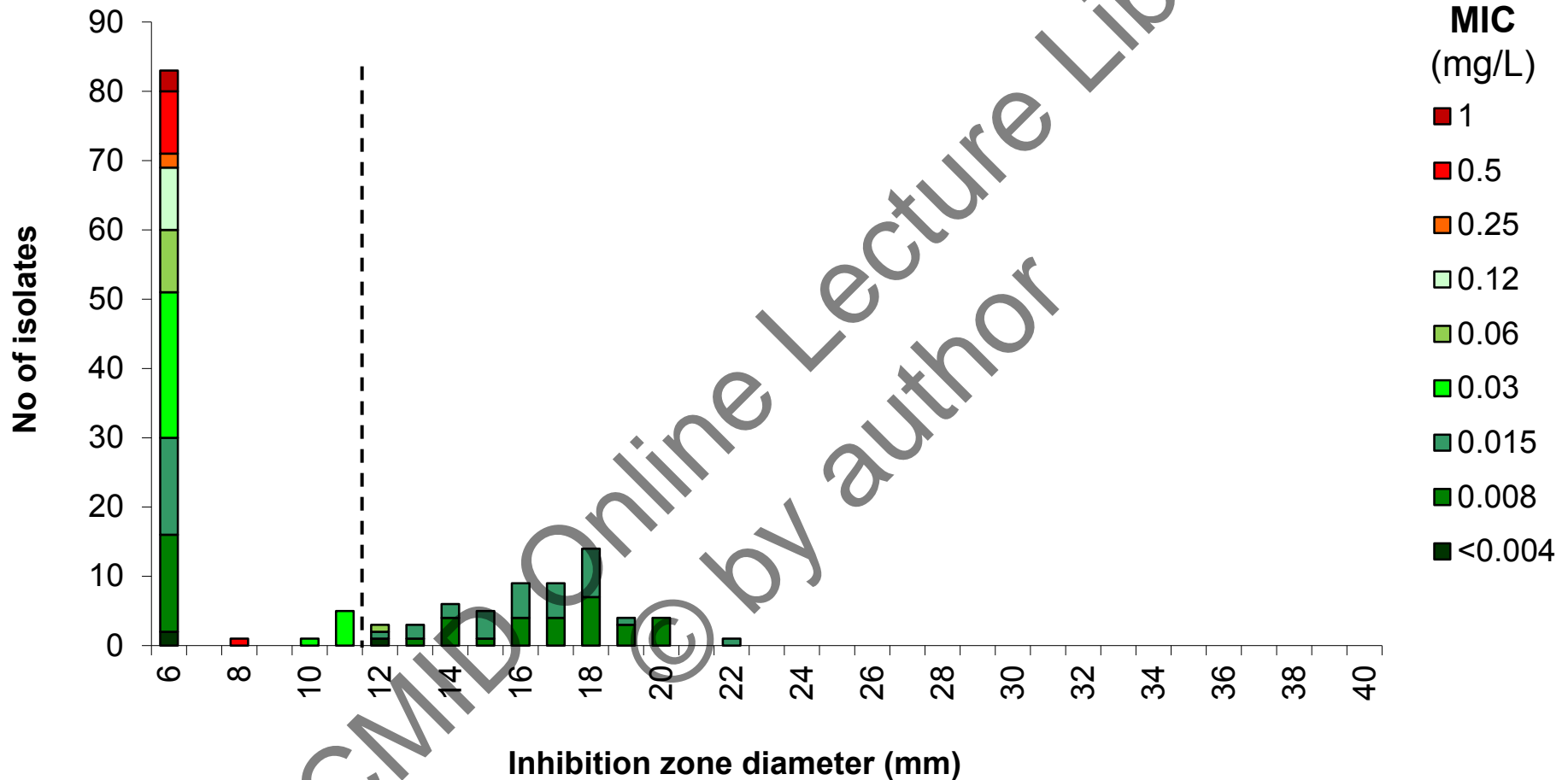
Breakpoints

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

ECOFF

WT ≤ 2 mg/L

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



Breakpoints

Cefotaxime MIC

Benzylpenicillin zone diameter (screen)

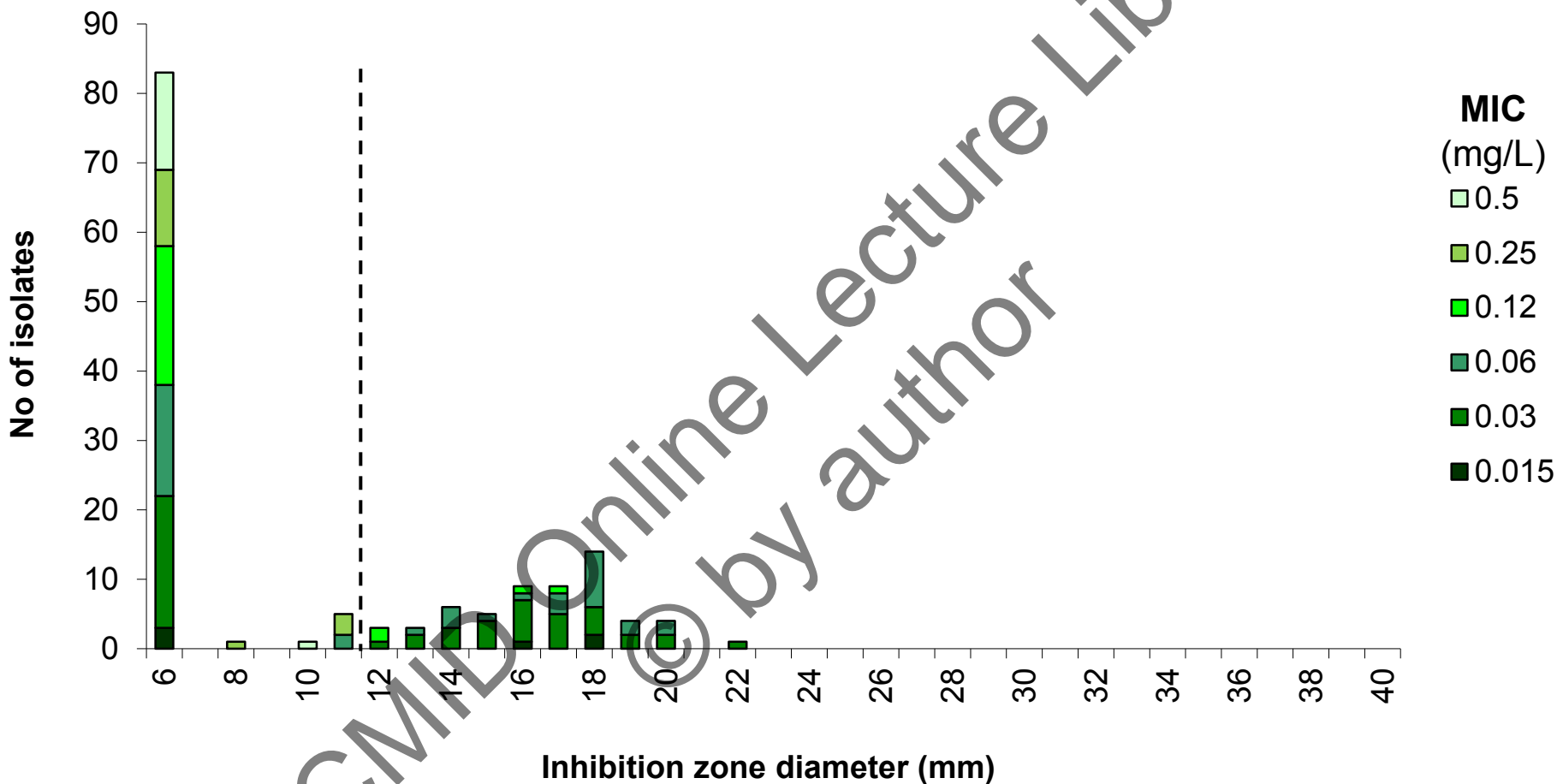
$S \leq 0.12$, $R > 0.12$ mg/L

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

ECOFF

WT ≤ 0.06 mg/L

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



Breakpoints

Meropenem MIC

Benzylpenicillin zone diameter (screen)

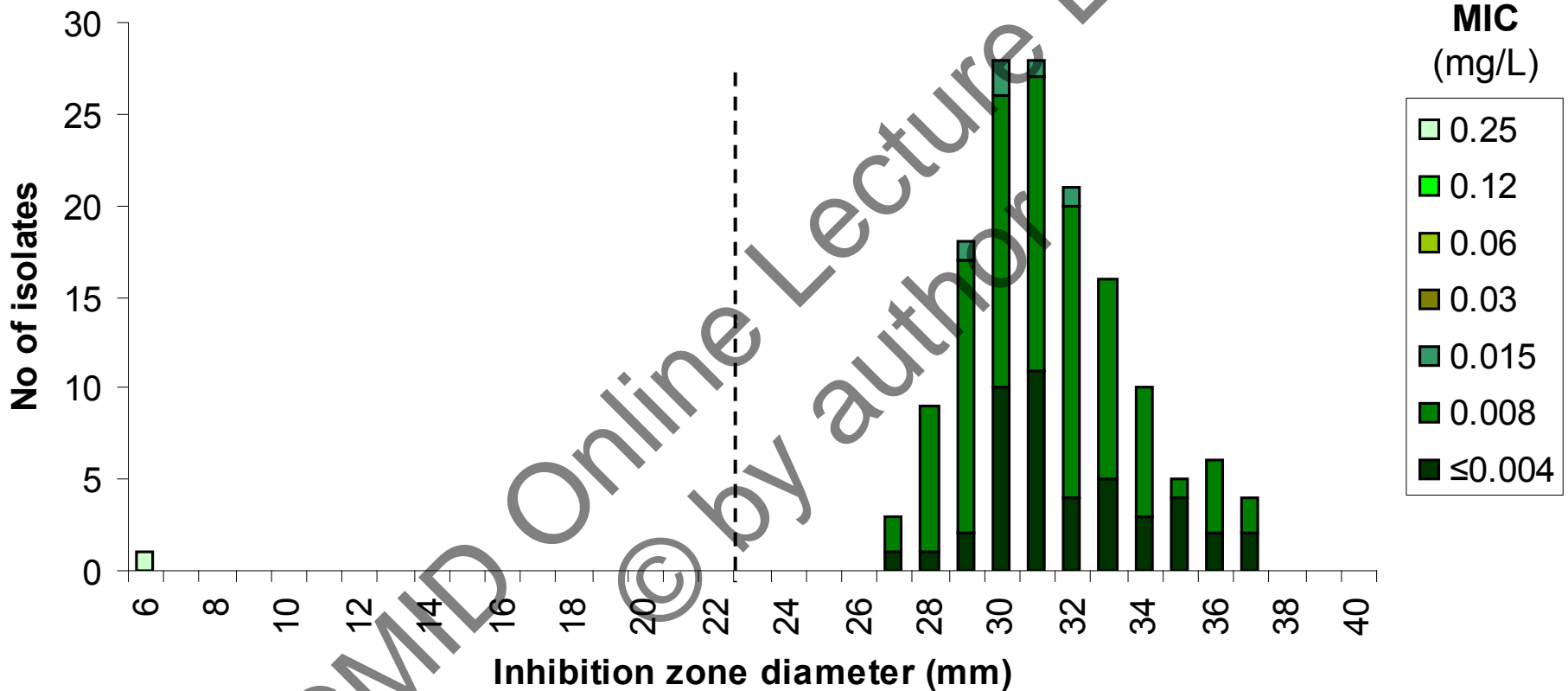
$S \leq 2$, $R > 2$ mg/L

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

ECOFF

$WT \leq 0.25$ mg/L

Nalidixic acid 30 µg vs. Ciprofloxacin MIC *H. influenzae*, 149 clinical isolates



Breakpoints

Ciprofloxacin MIC

Nalidixic acid zone diameter

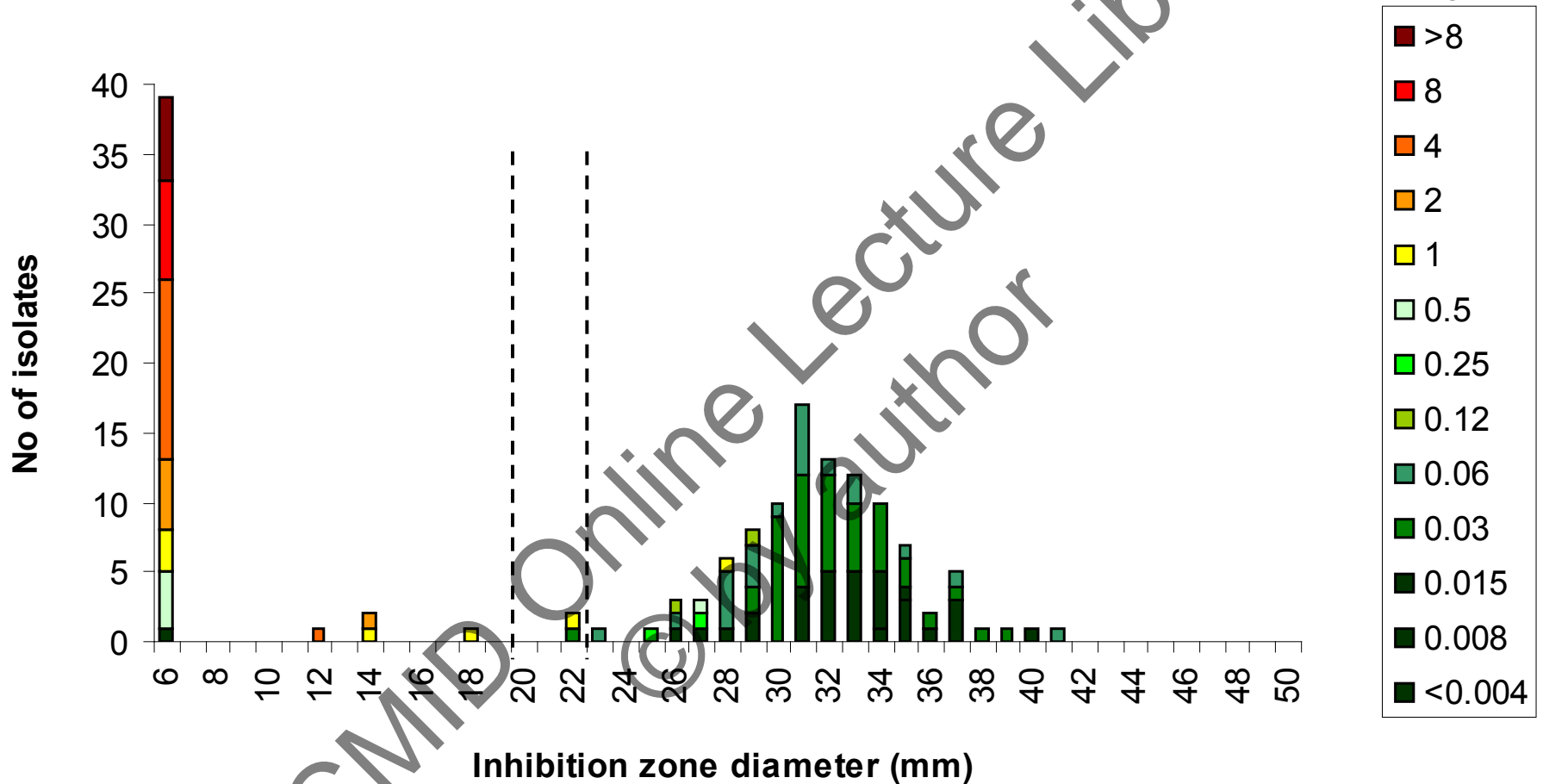
S ≤ 0.5, R > 0.5 mg/L

S ≥ 23, R < 23 mm

ECOFF

WT ≤ 0.06 mg/L

Trimethoprim-sulfamethoxazole 25 µg vs. MIC *H. influenzae*, 147 clinical isolates



Breakpoints		ECOFF
MIC	S ≤ 0.5, R > 1 mg/L	WT ≤ 0.5 mg/L
Zone diameter	S ≥ 23, R < 20 mm	



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Disk diffusion testing of
Campylobacter jejuni
&
Campylobacter coli

Erika Matuschek

Dik Mevius, Kees Veldman

Antti Hakanen, Mirva Lehtopolku

Gunnar Kahlmeter

2012

Q3

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EUCAST methodology

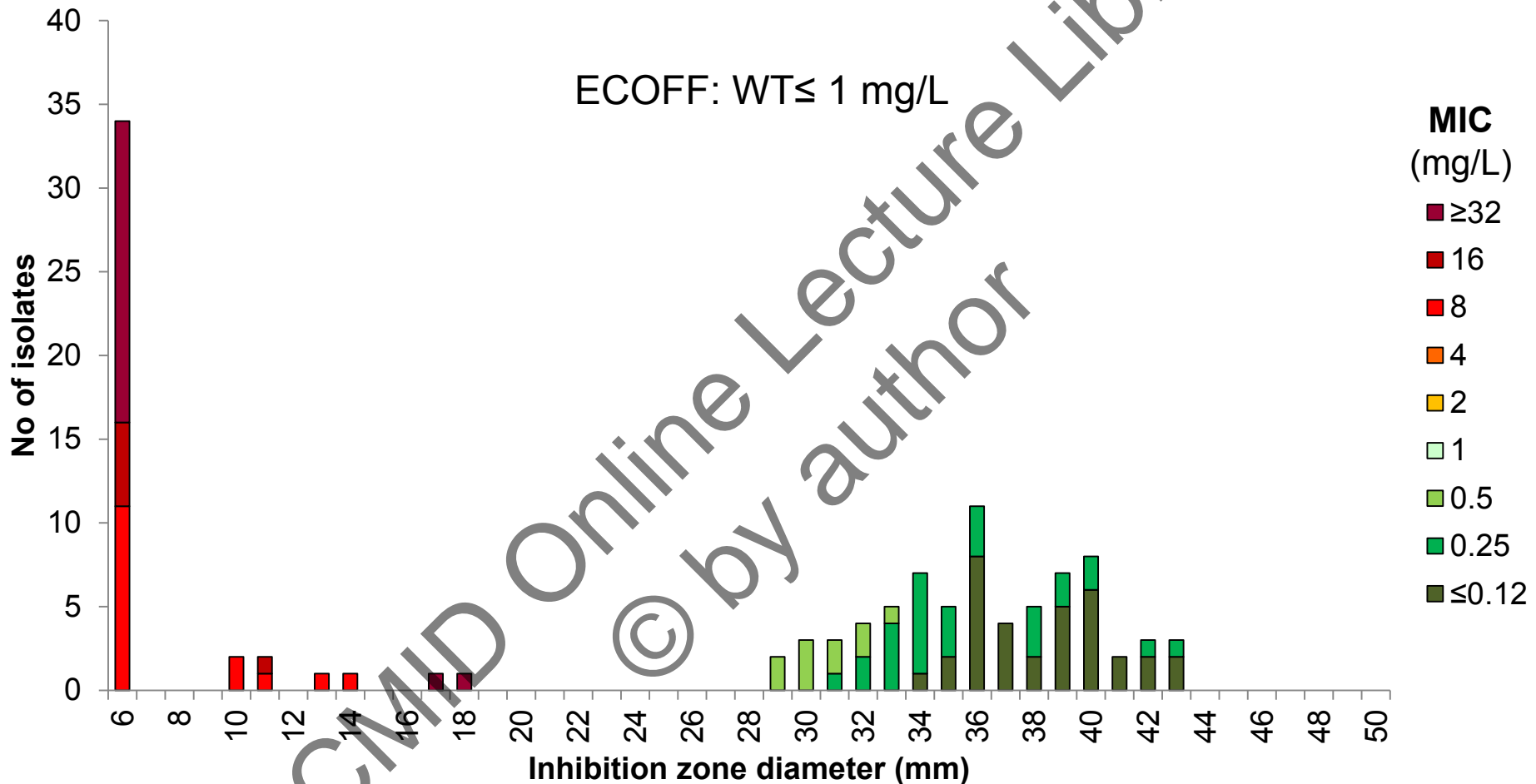
Media	MH-F (pre-dried plates to prevent swarming)
Inoculum	McFarland 0.5
Incubation	Microaerobic environment 41°C 24 h ± 30 min*
Reading	EUCAST standard reading

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

Validation of methodology

- Two collections of isolates with known MIC values (BMD)
 - NL collection (15 *C. jejuni* and 15 *C. coli*)
 - FI collection (17 *C. jejuni* and 12 *C. coli*, including “difficult” isolates)
- Data submission via EUCAST website
 - QC data from 15 labs
 - Clinical data from 2 labs (...more data to come!)

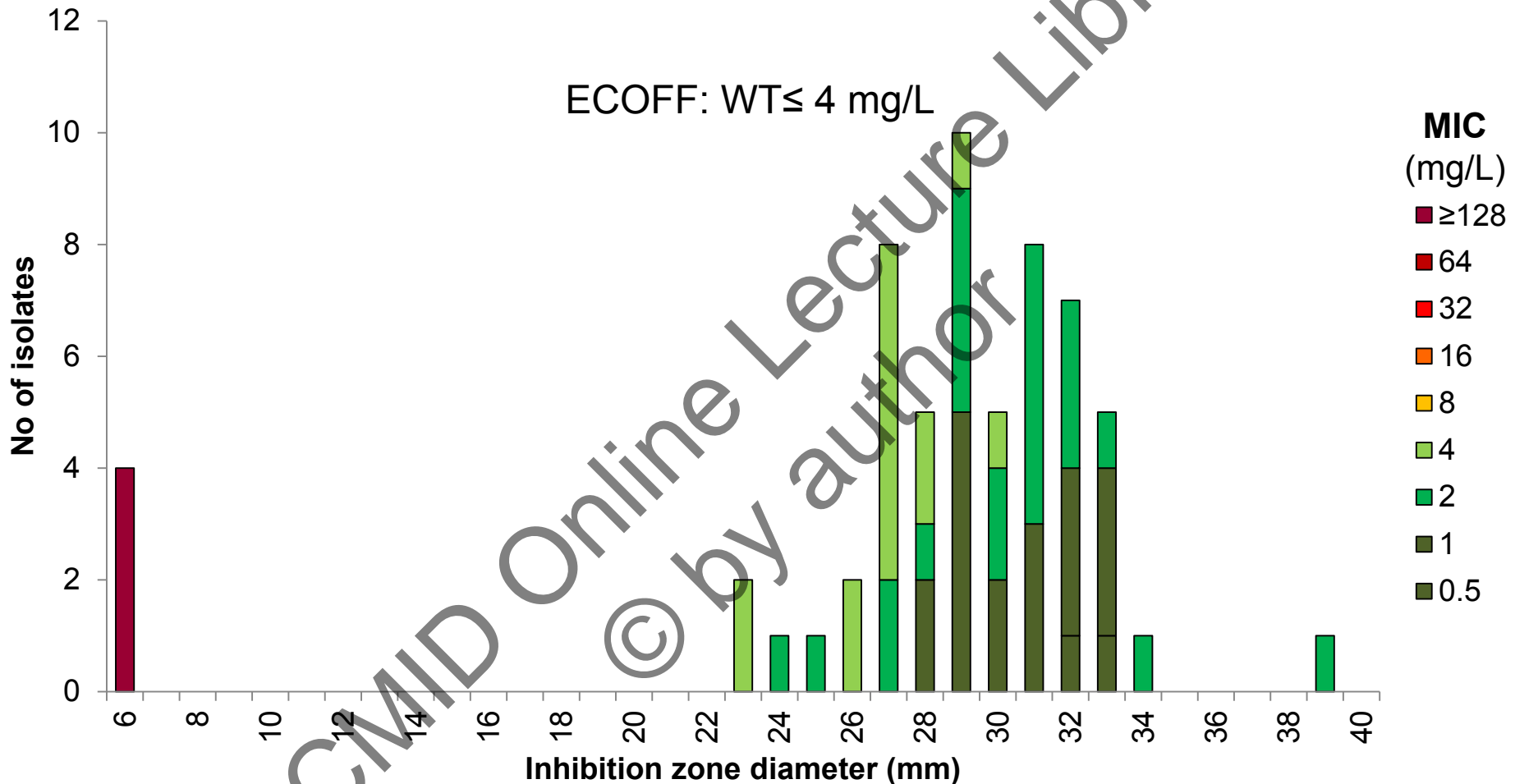
Ciprofloxacin 5 µg vs. MIC, Campylobacter jejuni and coli 57 clinical isolates tested in duplicate



NL and FI isolates read in Växjö.

All isolates tested in duplicate on in-house MH-F plates from Oxoid and BBL MH.

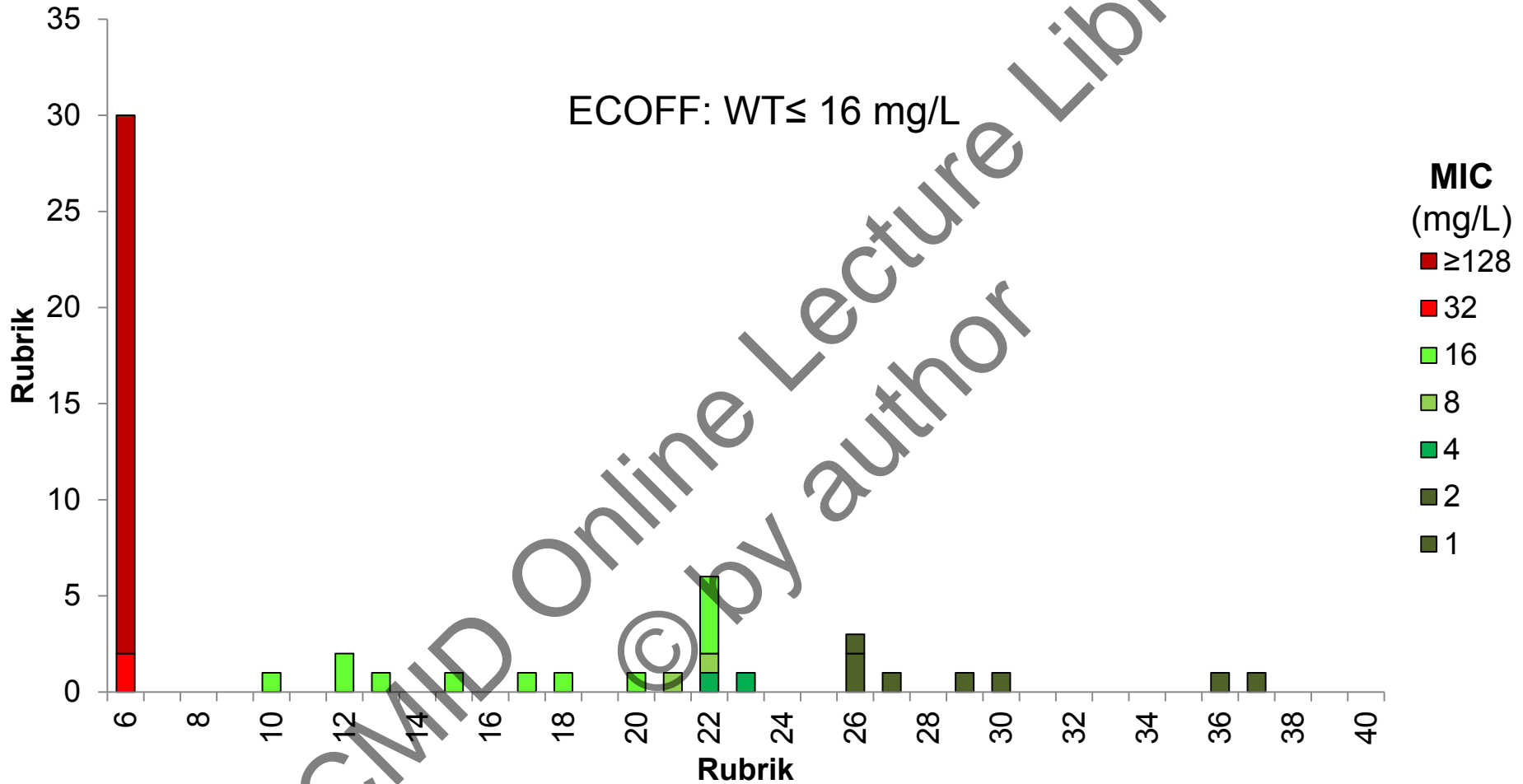
Erythromycin 15 µg vs. MIC, Campylobacter jejuni
30 clinical isolates tested in duplicate



NL and FI isolates read in Växjö.

All isolates tested in duplicate on in-house MH-F plates from Oxoid and BBL MH.

Erythromycin 15 µg vs. MIC, *Campylobacter coli*
27 clinical isolates tested in duplicate



NL and FI isolates read in Växjö.

All isolates tested in duplicate on in-house MH-F plates from Oxoid and BBL MH.

Pasteurella multocida

In collaboration with Dik Mevius, NL, and JMI Laboratories, Iowa, USA

- In-house isolates and isolates from NL
 - 131 clinical isolates from Sweden, Sentry and The Netherlands.

MICs and zone diameters by EUCAST

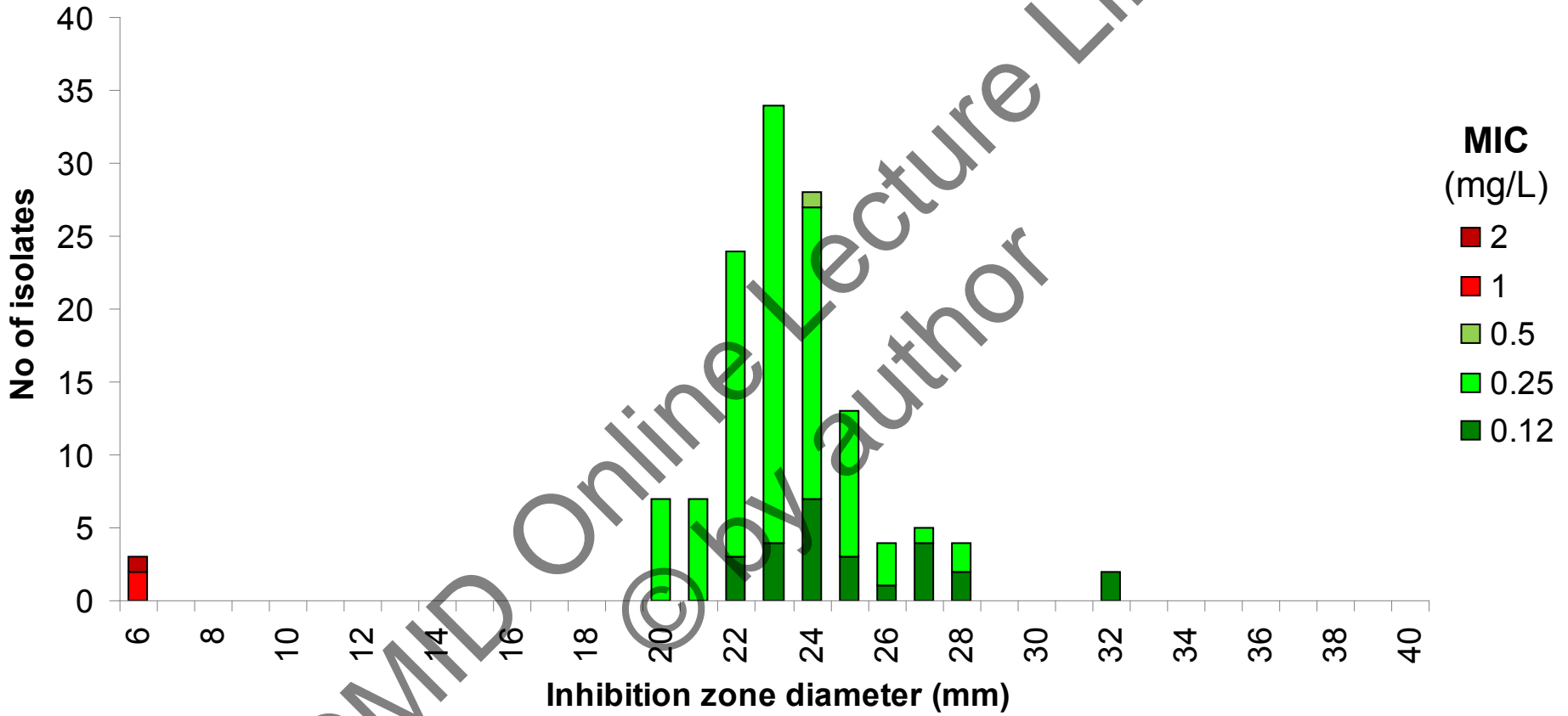
Disk diffusion on MH-F, ambient air, 35 C, 16 – 20 h

Pasteurella multocida

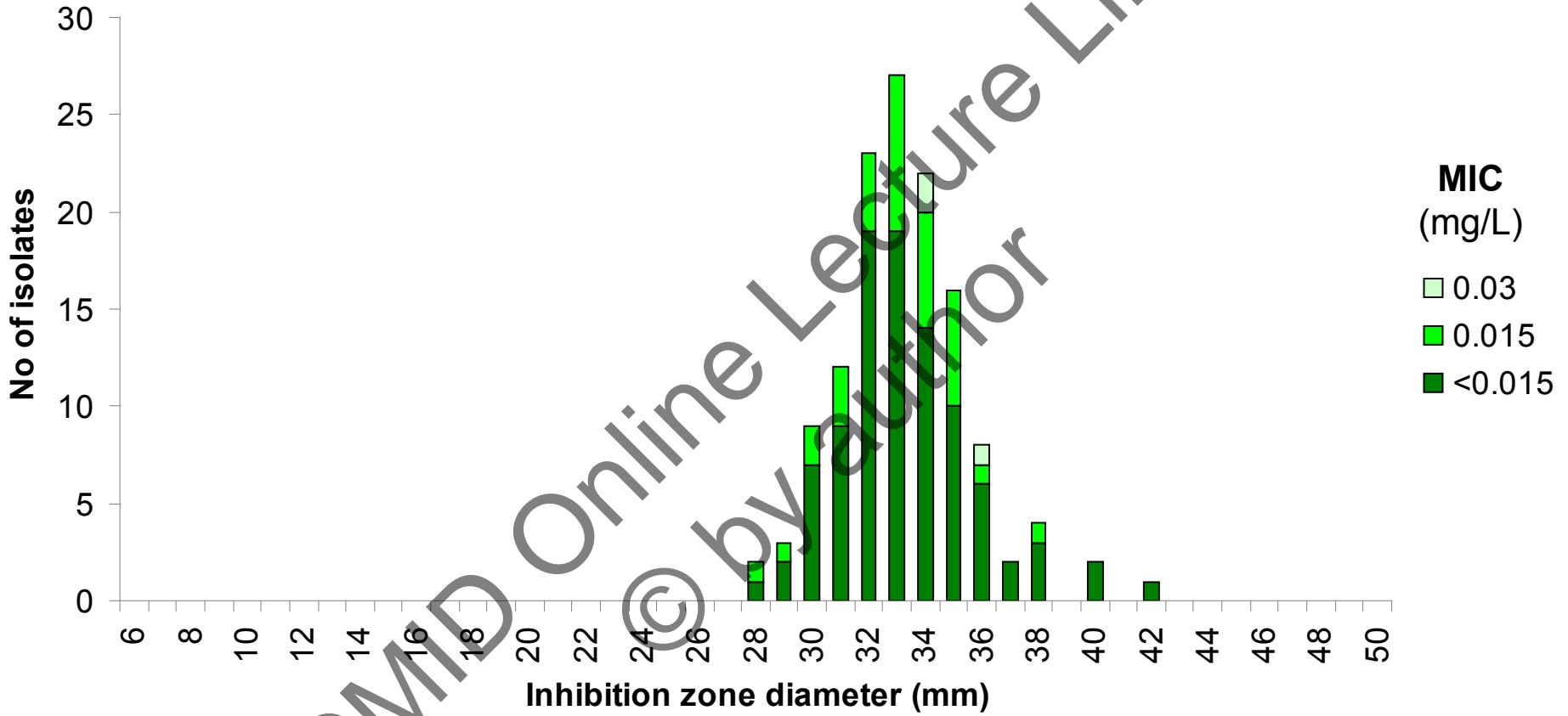
Antibiotics tested

- Benzylpenicillin
- Ampicillin
- Amoxicillin
- Amoxicillin-clavulanic acid
- Cefotaxime
- Ciprofloxacin
- Tetracycline
- Trimethoprim-sulfamethoxazole

Ampicillin 2 µg vs. MIC
Pasteurella multocida, 131 clinical isolates

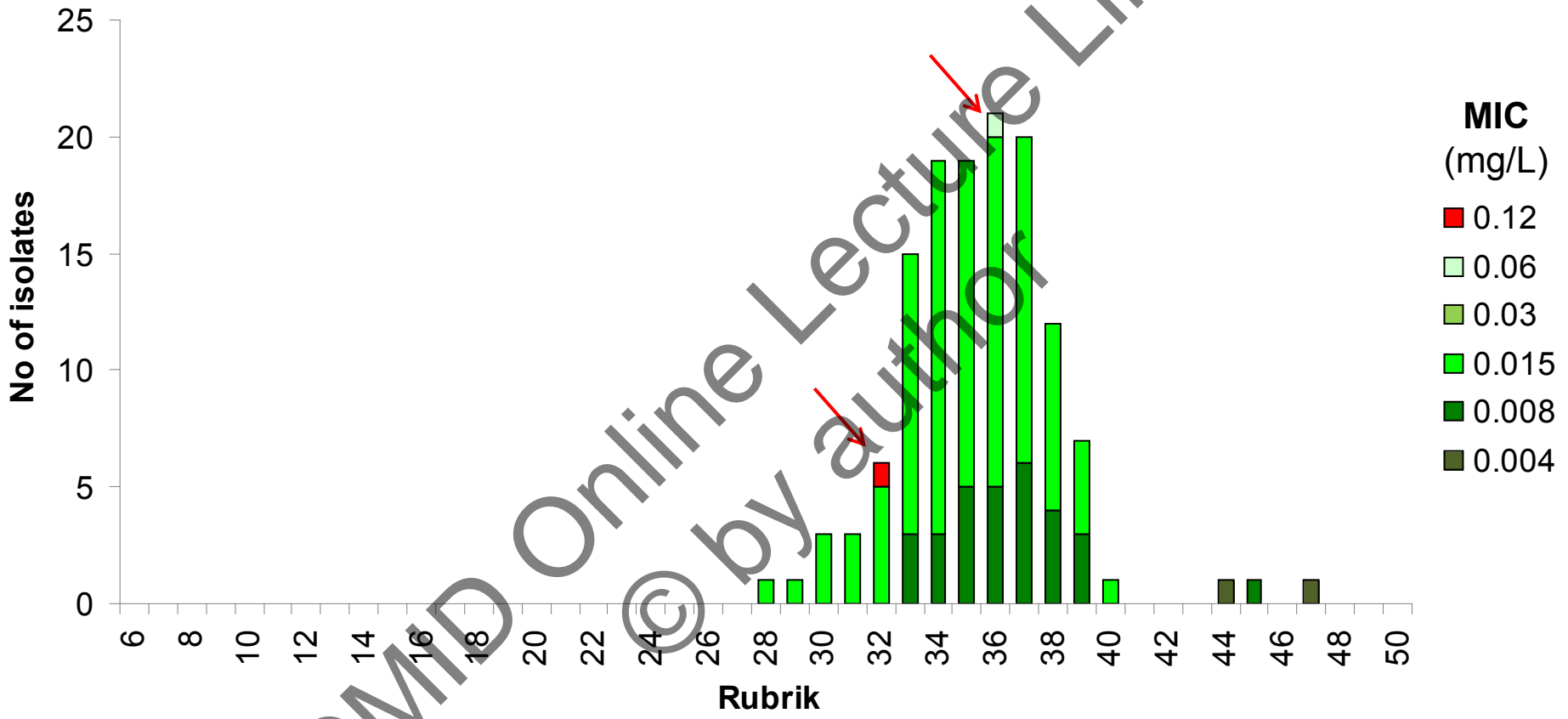


Cefotaxime 5 μ g vs. MIC
***Pasteurella multocida*, 131 clinical isolates**

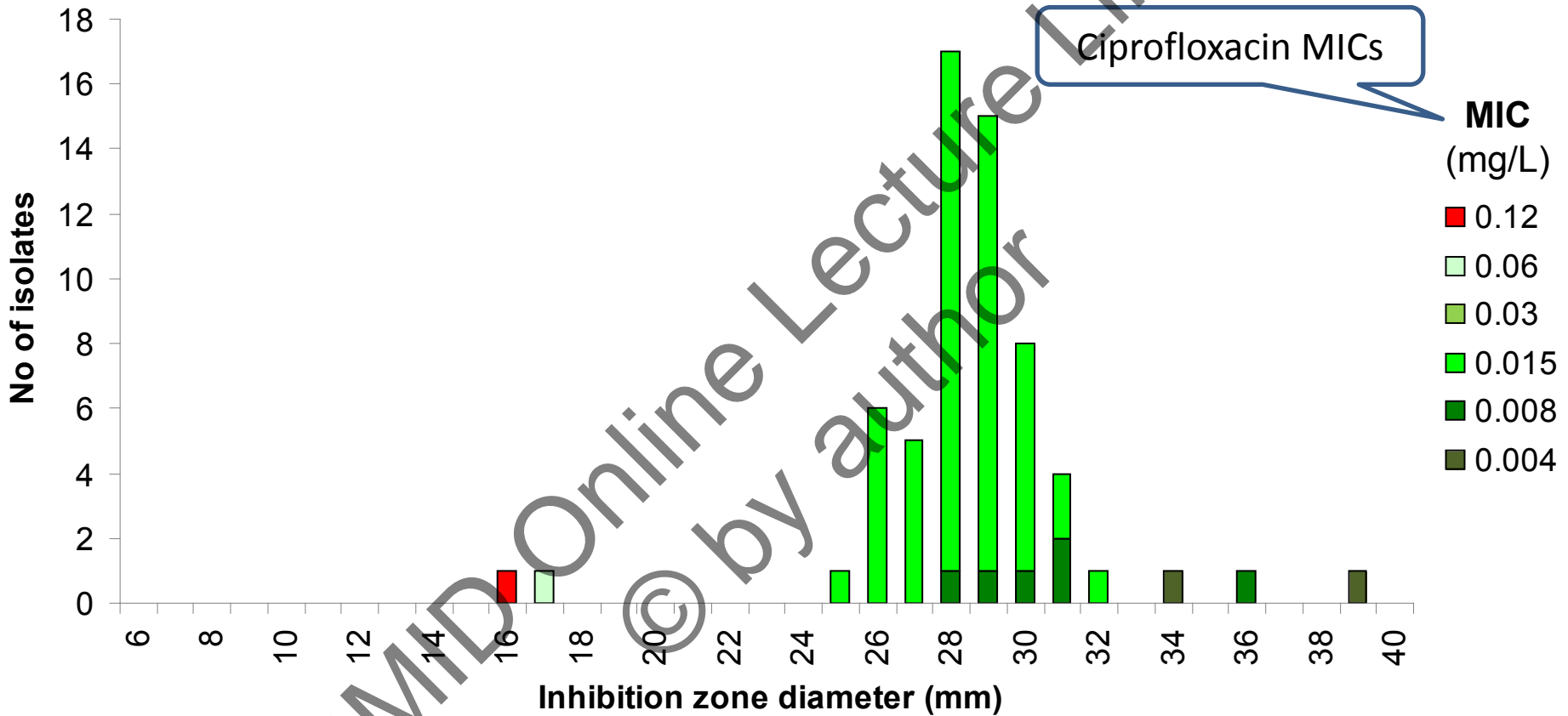


Ciprofloxacin 5 µg vs. MIC

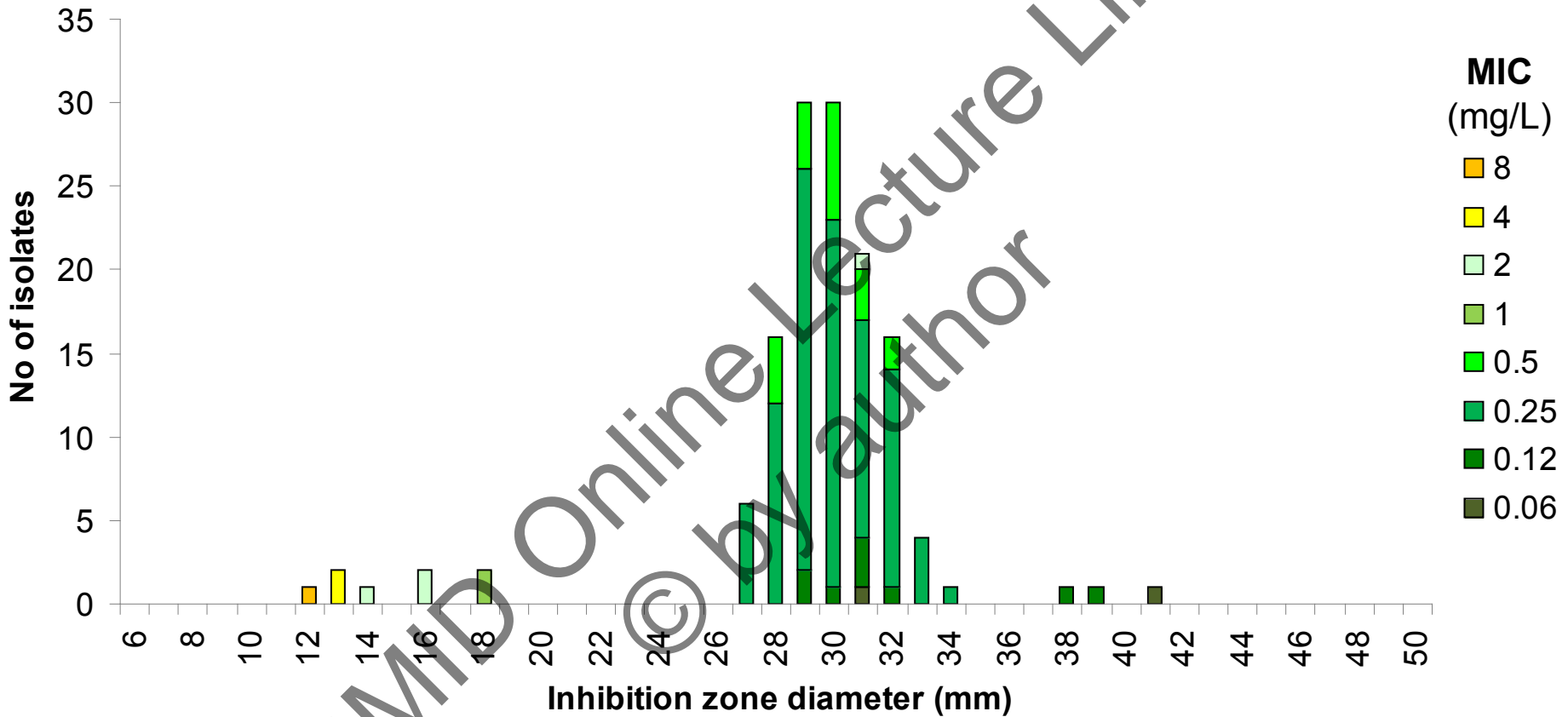
Pasteurella multocida, 131 clinical isolates



Naldixic acid 30 µg vs. Ciprofloxacin MIC *Pasteurella multocida*, 62 clinical isolates



Tetracycline 30 µg vs. Doxycycline MIC
***Pasteurella multocida*, 135 clinical isolates**

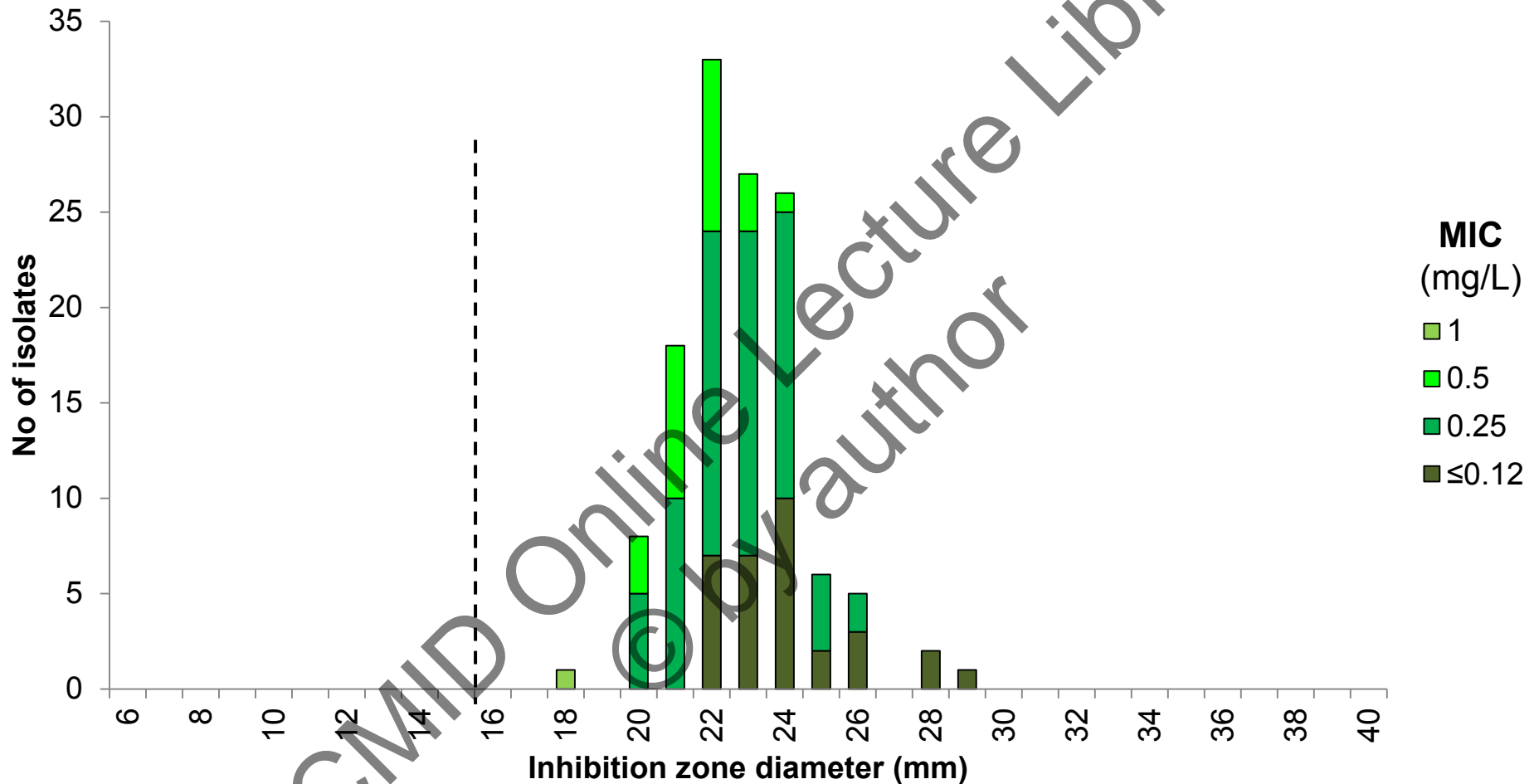


Listeria monocytogenes

- 129 clinical isolates collected from 5 test sites:
 - Denmark
 - Israel
 - Norway
 - Sweden
 - United Kingdom
- Disk diffusion performed on all isolates at all sites
- MIC determination performed with broth microdilution and gradient tests on MH-F media at one site (Karen Bowker, Bristol, UK)

Ampicillin 2 µg vs. MIC

Listeria monocytogenes, 127 clinical isolates



Breakpoints

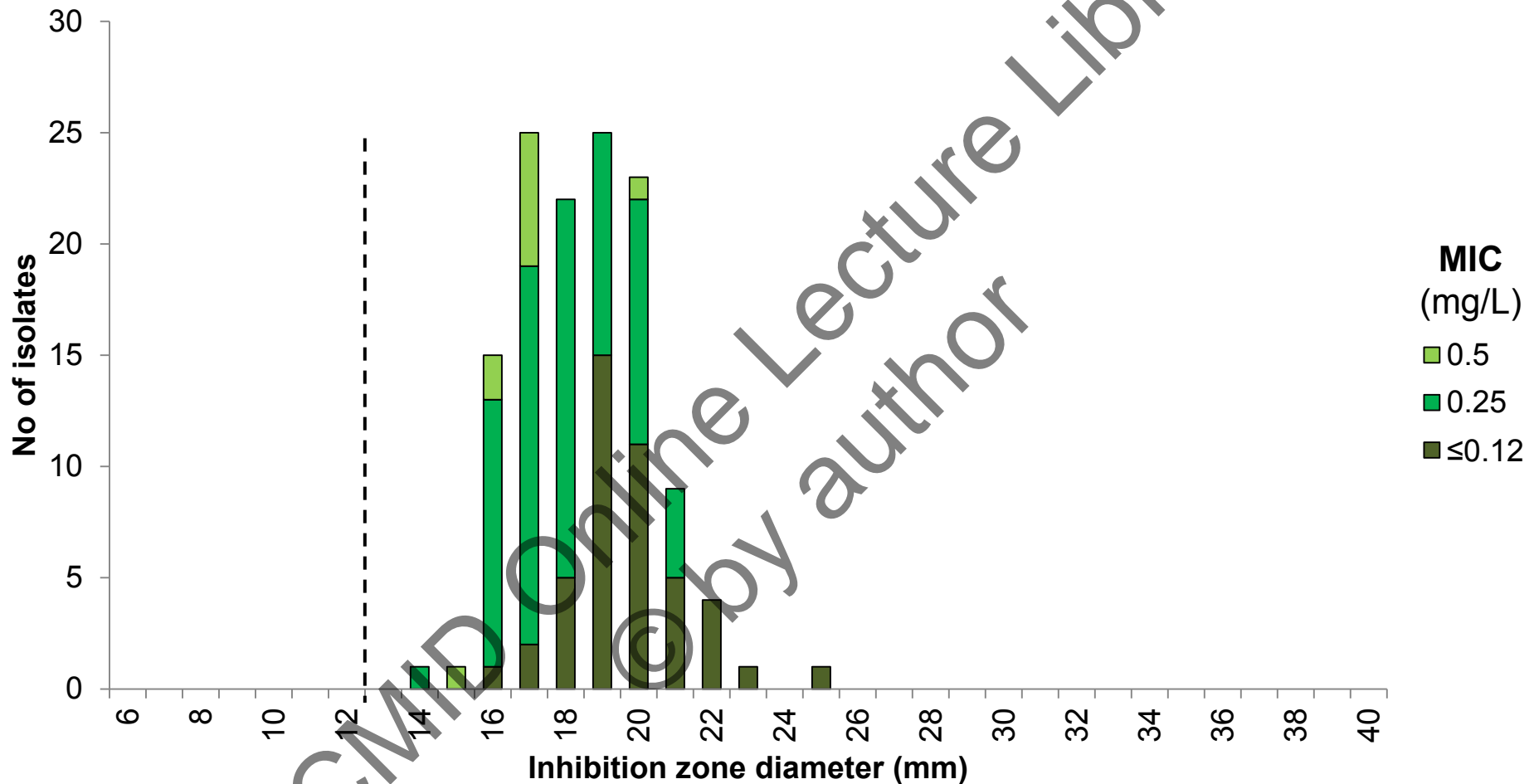
MIC S ≤ 1, R > 1 mg/L
 Zone diameter S ≥ 16, R < 16 mm

ECOFF

WT ≤ 2 mg/L

Benzylpenicillin 1 unit vs. MIC

Listeria monocytogenes, 127 clinical isolates



Breakpoints

MIC

S ≤ 1, R > 1 mg/L

Zone diameter

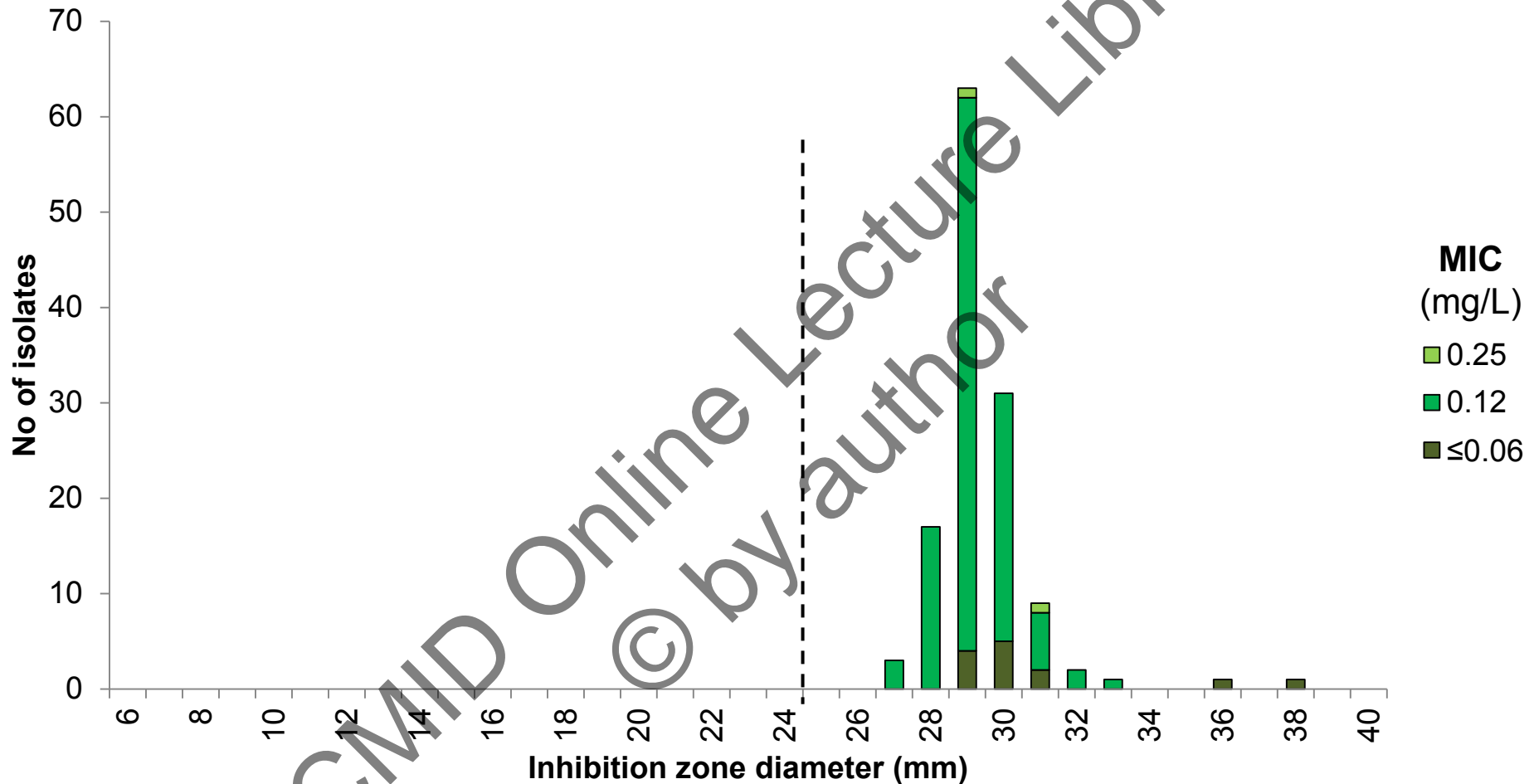
S ≥ 13, R < 13 mm

ECOFF

WT ≤ 1 mg/L

Erythromycin 15 µg vs. MIC

Listeria monocytogenes, 128 clinical isolates



Breakpoints

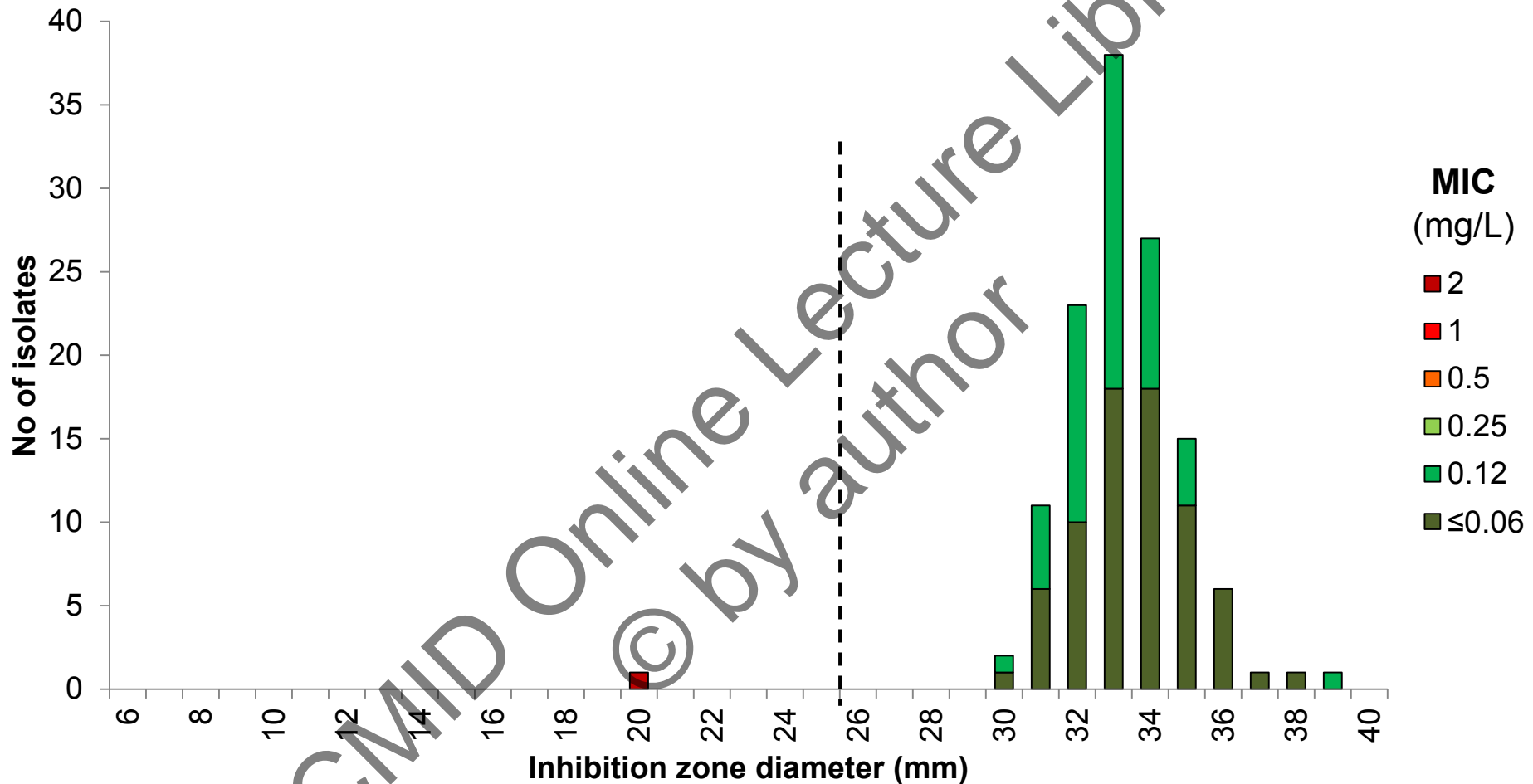
MIC S ≤ 1, R > 1 mg/L
 Zone diameter S ≥ 25, R < 25 mm

ECOFF

WT ≤ 1 mg/L

Meropenem 10 µg vs. MIC

Listeria monocytogenes, 126 clinical isolates



Breakpoints

MIC

S ≤ 0.25, R > 0.25 mg/L

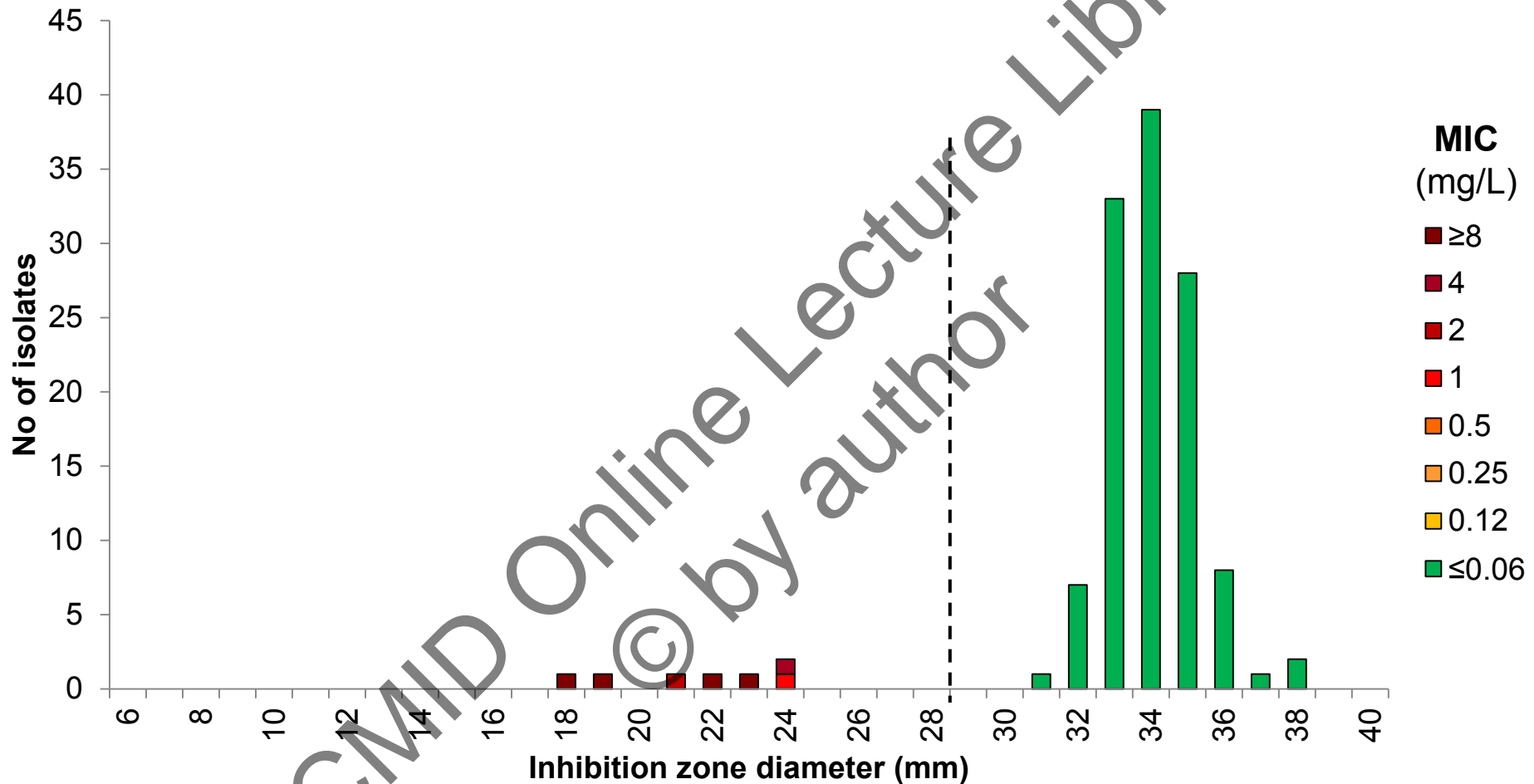
Zone diameter

S ≥ 26, R < 26 mm

ECOFF

WT ≤ 0.25 mg/L

Trimethoprim-sulfamethoxazole 1.25-23.75 µg vs. MIC *Listeria monocytogenes*, 126 clinical isolates



Breakpoints

MIC S ≤ 0.06, R > 0.06 mg/L

Zone diameter S ≥ 29, R < 29 mm

Corynebacterium spp.

- A collection of 259 species-identified (Maldi-tof) *Corynebacterium* spp. from Spain, Sweden and USA (SENTRY)
- Disk diffusion and MIC (BMD with MH-F broth)
 - Isolates with non-sufficient growth after 16-20 h incubation was re-incubated and read after a total of 40-44 h incubation

Corynebacterium spp.

Antibiotics tested

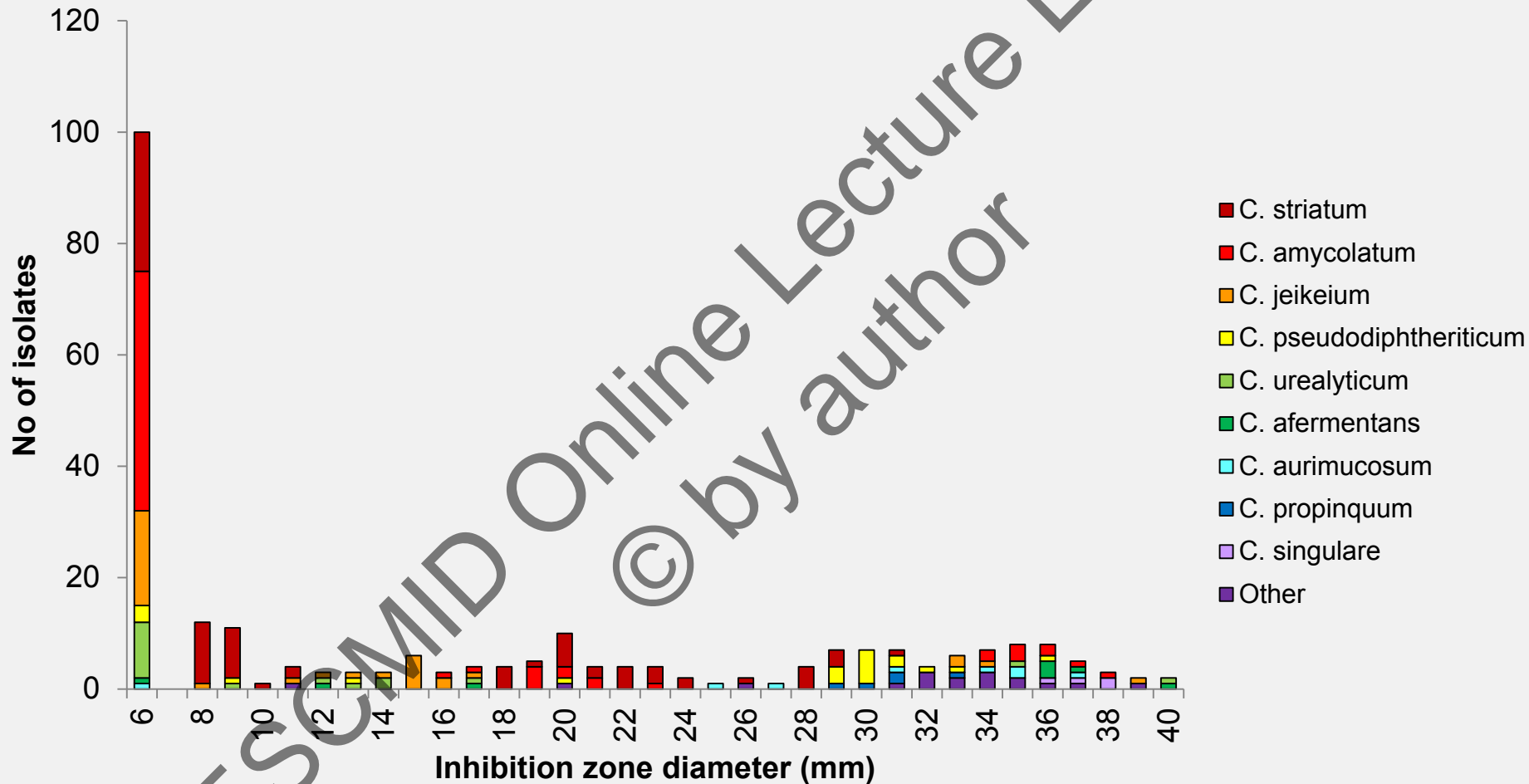
- Benzylpenicillin
- Ciprofloxacin
- Moxifloxacin
- Gentamicin
- Vancomycin
- Tetracycline
- Clindamycin
- Linezolid
- Rifampicin
- Trimethoprim-sulfamethoxazole

Preliminary report

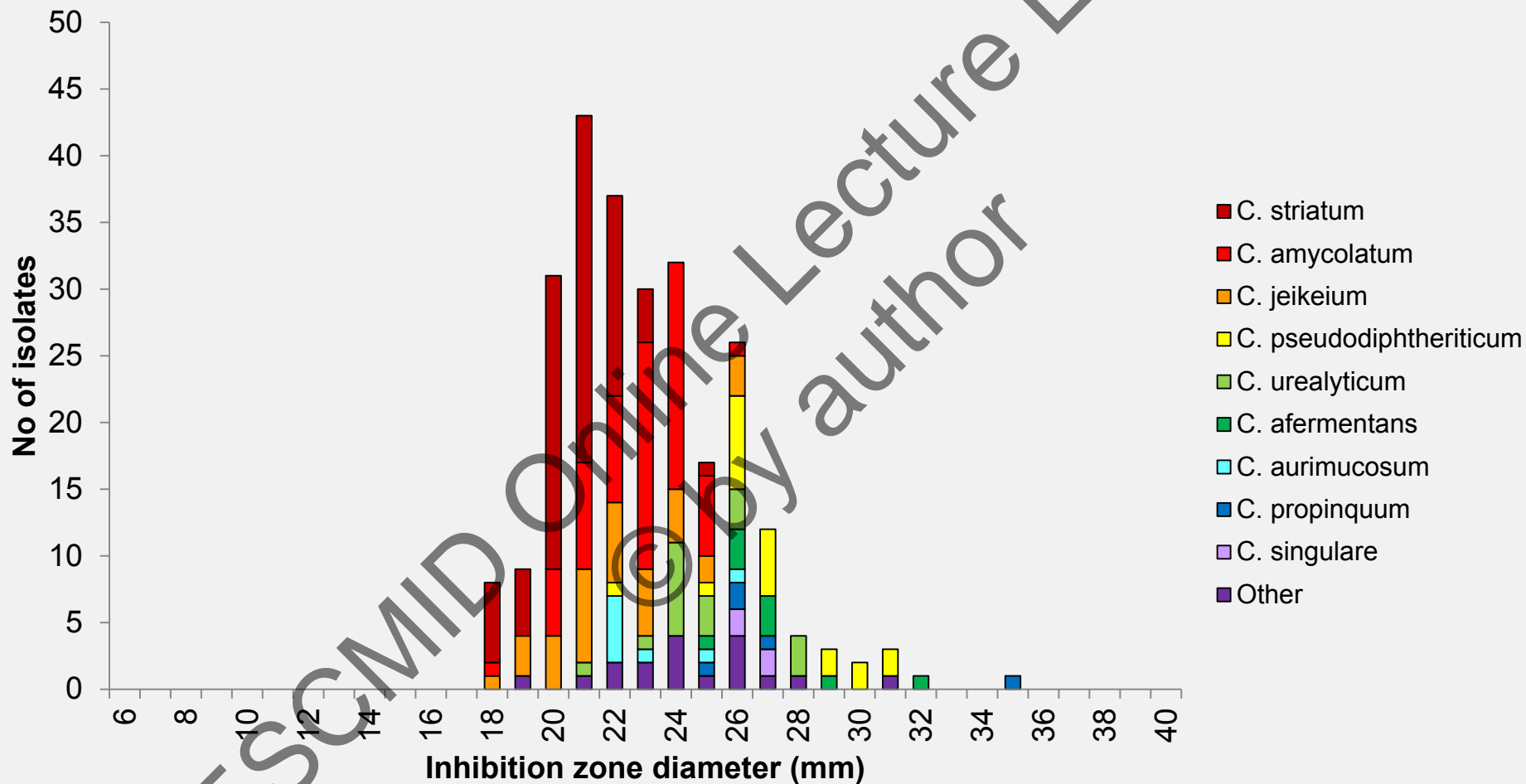
See today's poster session for more information

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Moxifloxacin 5 μ g vs. species *Corynebacterium* spp., 257 clinical isolates



Vancomycin 5 µg vs. species *Corynebacterium* spp., 259 clinical isolates



Fastidious organisms

Summary

MH-F disk diffusion:

- ✓ *Haemophilus influenzae* v 2.0 (Jan 2012)
- ✓ *Moraxella catarrhalis* v 2.0 (Jan 2012)
- ✓ Streptococci A, B, C, G v 2.0 (Jan 2012)
- ✓ Viridans group streptococci v 2.0 (Jan 2012)
- ✓ *Streptococcus pneumoniae* v 2.0 (Jan 2012)
- ✓ *Listeria* v 2.0 (Jan 2012)

Campylobacter v 3.0 (Jan 2013)

Corynebacterium v 3.0 (Jan 2013)

Pasteurella v 3.0 (Jan 2013)

Other media (MIC breakpoints are available in v 2.0):

Clostridium difficile v 3.0 (Jan 2013)

Neisseria gonorrhoeae (2013)

Saturday 15.30 – 16.30

P671 Adjusting EUCAST zone diameter breakpoints for *Haemophilus influenzae* on the Mueller-Hinton fastidious media. J. Åhman*, E. Matuschek, P.R. Rhomberg, R.N. Jones, G. Kahlmeter

P676 Antimicrobial susceptibility testing of *Listeria monocytogenes* with EUCAST breakpoints: a multi-laboratory study. K. Bowker, J. Åhman, O. Natås, I. Nissan, P. Littauer, E. Matuschek*

P677 Susceptibility testing of ten antibiotics against *Corynebacterium* spp. Determined by broth microdilution, Etest and EUCAST disc diffusion methods. C. Salas*, C. Karlsson, A. Akerlund, C. Rodríguez-Mirones, E. Matuschek, L. Martínez-Martínez, G. Kahlmeter

P680 The *Brucella* blood agar for disc diffusion antimicrobial susceptibility testing – reproducibility results for *Clostridium difficile* ATCC 700057. U.S. Justesen, L.T. Erikstrup*, T.K. Danielsen, E. Matuschek, G. Kahlmeter

P682 EUCAST standardised disc diffusion methodology for *Campylobacter jejuni* and *C. coli*. E. Matuschek*, K. Veldman, A. Hakanen, S. Bengtsson, M. Lehtopolku, D. Mevius, G. Kahlmeter

Sunday 12.30 – 13.30

P1092 Antimicrobial susceptibility and species identification of *Corynebacterium* spp. strains collected in Europe and USA medical centres, 2006–2010. H. Sader*, P. Rhomberg, R. Jones, J. Åhman, E. Matuschek, G. Kahlmeter

Other EUCAST posters

P675 Contemporary doxycycline and tetracycline susceptibility testing using CLSI and EUCAST criteria for Gram-positive pathogens: results from SENTRY programme. *R. Jones*, M. Stilwell (North Liberty, US)*

P684 Comparison of Neo-sensitabs (ROSCO) tablets with paper discs (OXOID) for antimicrobial susceptibility testing of Gram-negative clinical isolates according to the EUCAST recommendations
H. Rodriguez-Villalobos, A. Boeras (Brussels, BE)*

P 686 New EUCAST breakpoint for detection of antimicrobial susceptibility in *Helicobacter pylori*
T. Alarcon, A. Somodevilla, M. (Madrid, ES)*

Acknowledgements

- Erika Matuschek, Jenny Åhman, Anna Petersson, Stina Bengtsson in Växjö, Sweden
- Derek Brown, scientific secretary of EUCAST
- Karen Bowker (*Listeria*)
- Luis Martinez-Martinez (*Corynebacterium* spp.)
- JMI Laboratories – especially Ron Jones, Helio Sader, Paul Rhomberg (*H. influenzae*, *Corynebacterium* spp., *Pasteurella multocida*)
- ...and many others