



News from EUCAST Development Laboratory (EDL)

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New antimicrobial agents

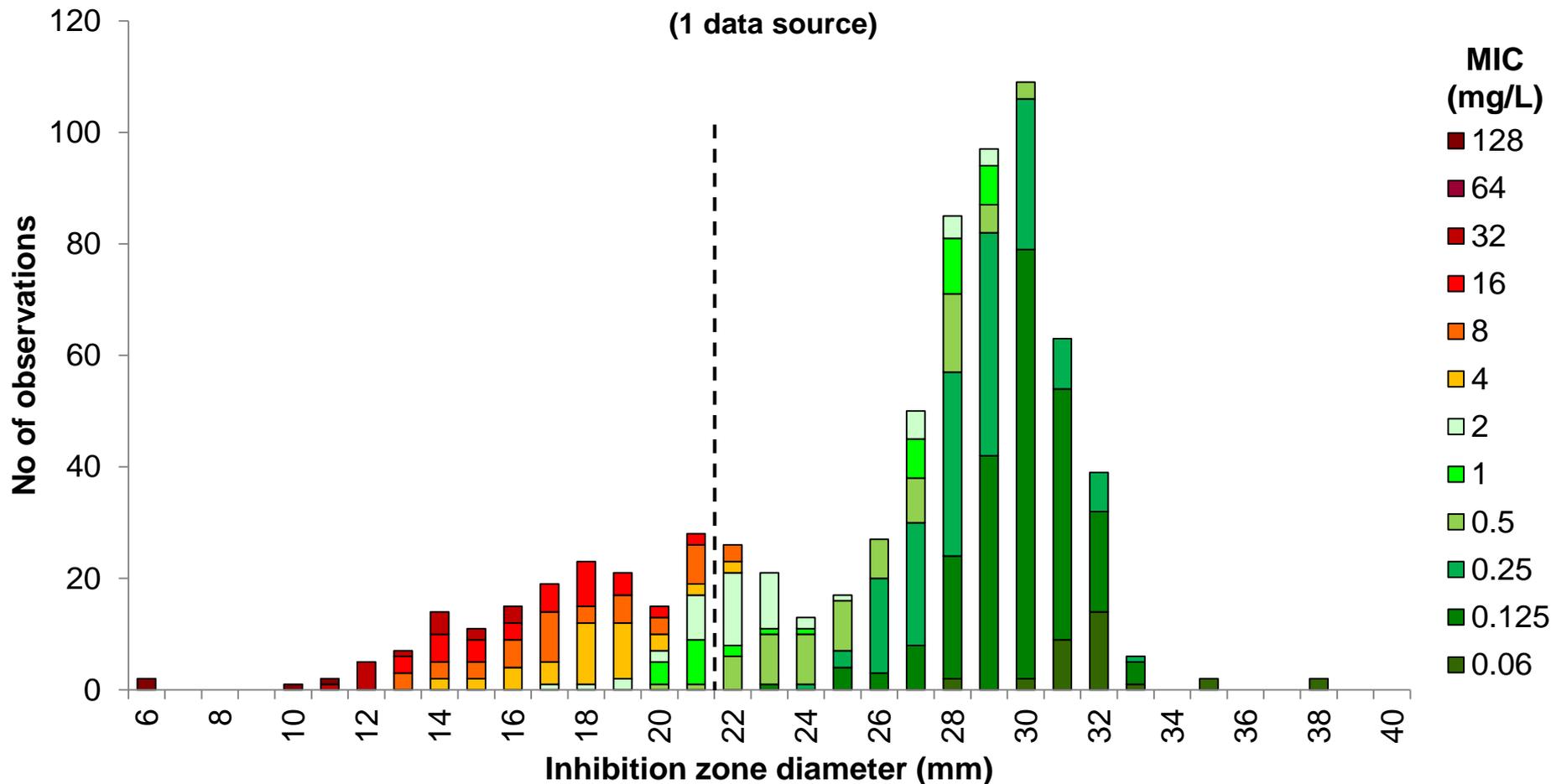
- Development of zone diameter breakpoints and disk QC criteria for new antimicrobial agents.
 - Disk diffusion methods are important for new agents since a method based on disks can be made commercially available long before many other systems.
- SOP for developing an optimal disk potency for EUCAST and CLSI (CLSI-EUCAST joint working group)
 - Submissions to joint working group
 - 4 agents ongoing
 - Phase 1: 10 potencies varying from very low to very high
 - Phase 2: Additional tests for 2-4 selected potencies

Recent EUCAST criteria for new agents

- Imipenem-relebactam
 - *Enterobacterales* and *P. aeruginosa*
 - *Acinetobacter* spp. (no effect of relebactam)
- Meropenem-vaborbactam
 - *Enterobacterales* and *P. aeruginosa*
- Cefiderocol
 - *Enterobacterales* and *P. aeruginosa*
 - Cut-off values corresponding to PK-PD breakpoints for *Acinetobacter* spp. and *S. maltophilia*

Imipenem-relebactam 10-25 µg vs. MIC *Enterobacterales*, 180 isolates (720 correlates)

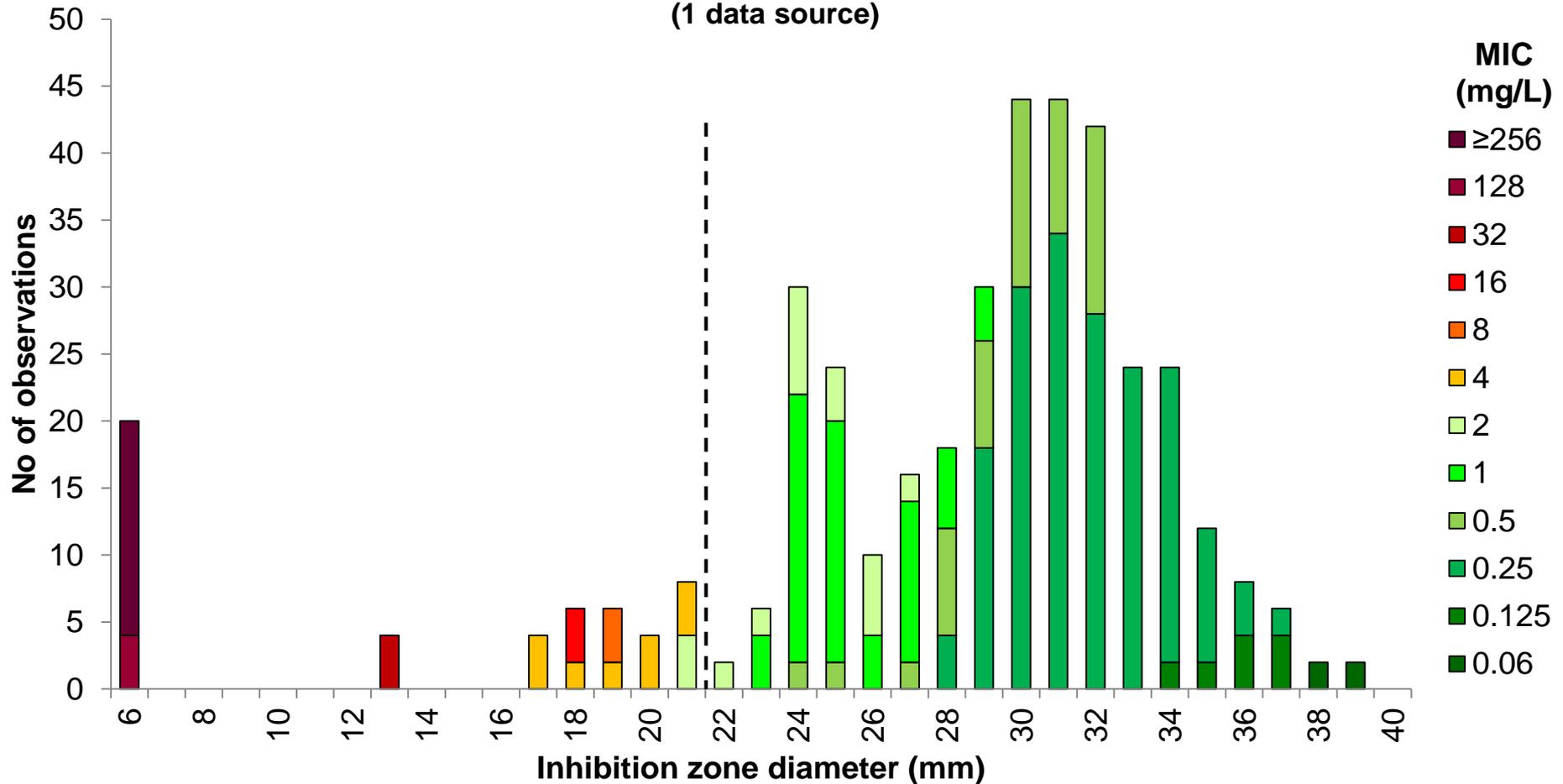
(1 data source)



Breakpoints	
MIC	S ≤ 2, R > 2 mg/L
Zone diameter	S ≥ 22, R < 22 mm

Imipenem-relebactam 10-25 µg vs. MIC *P. aeruginosa*, 99 isolates (396 correlates)

(1 data source)

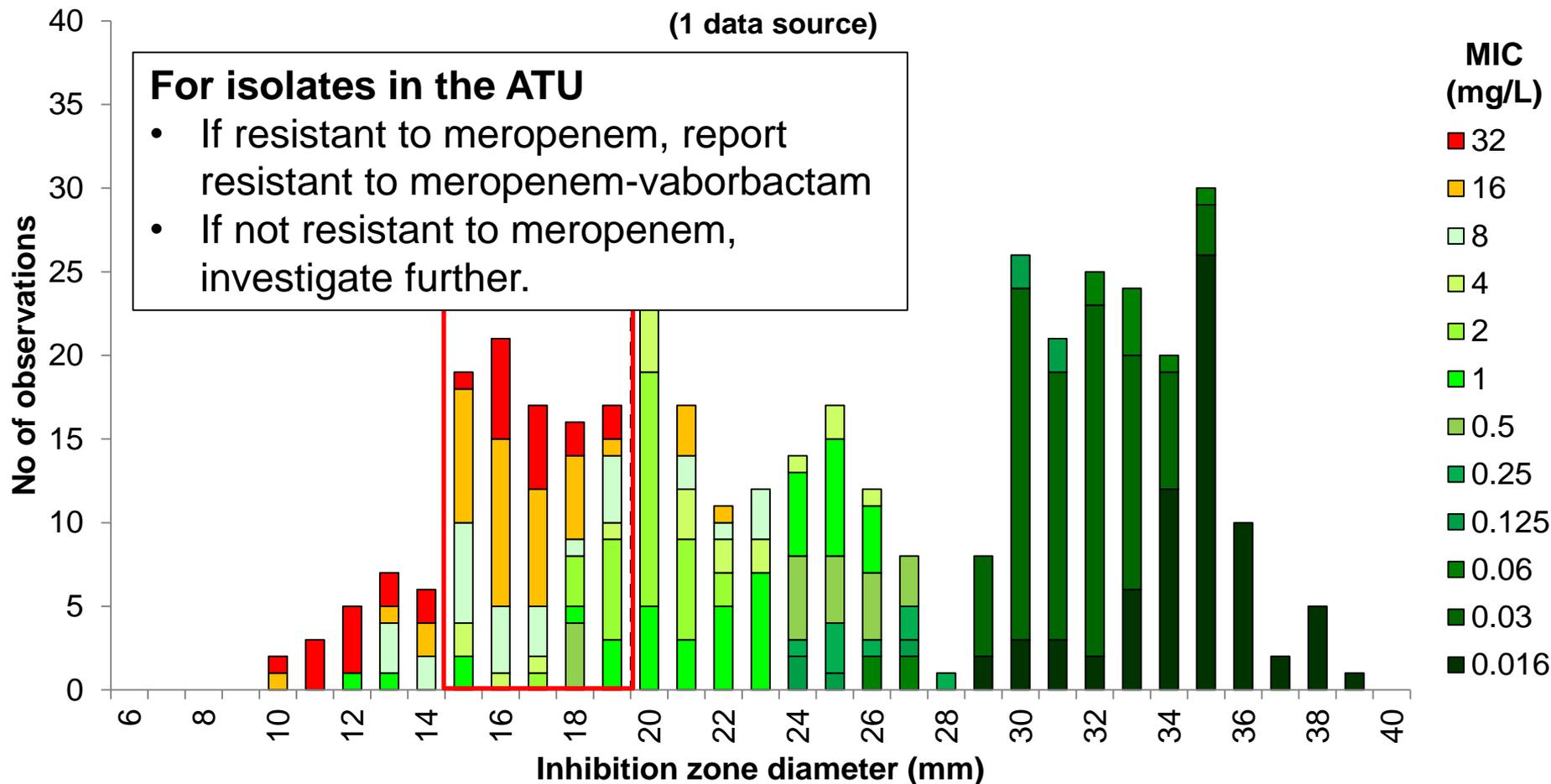


Breakpoints

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

Zone diameter $S \geq 22, R < 22$ mm

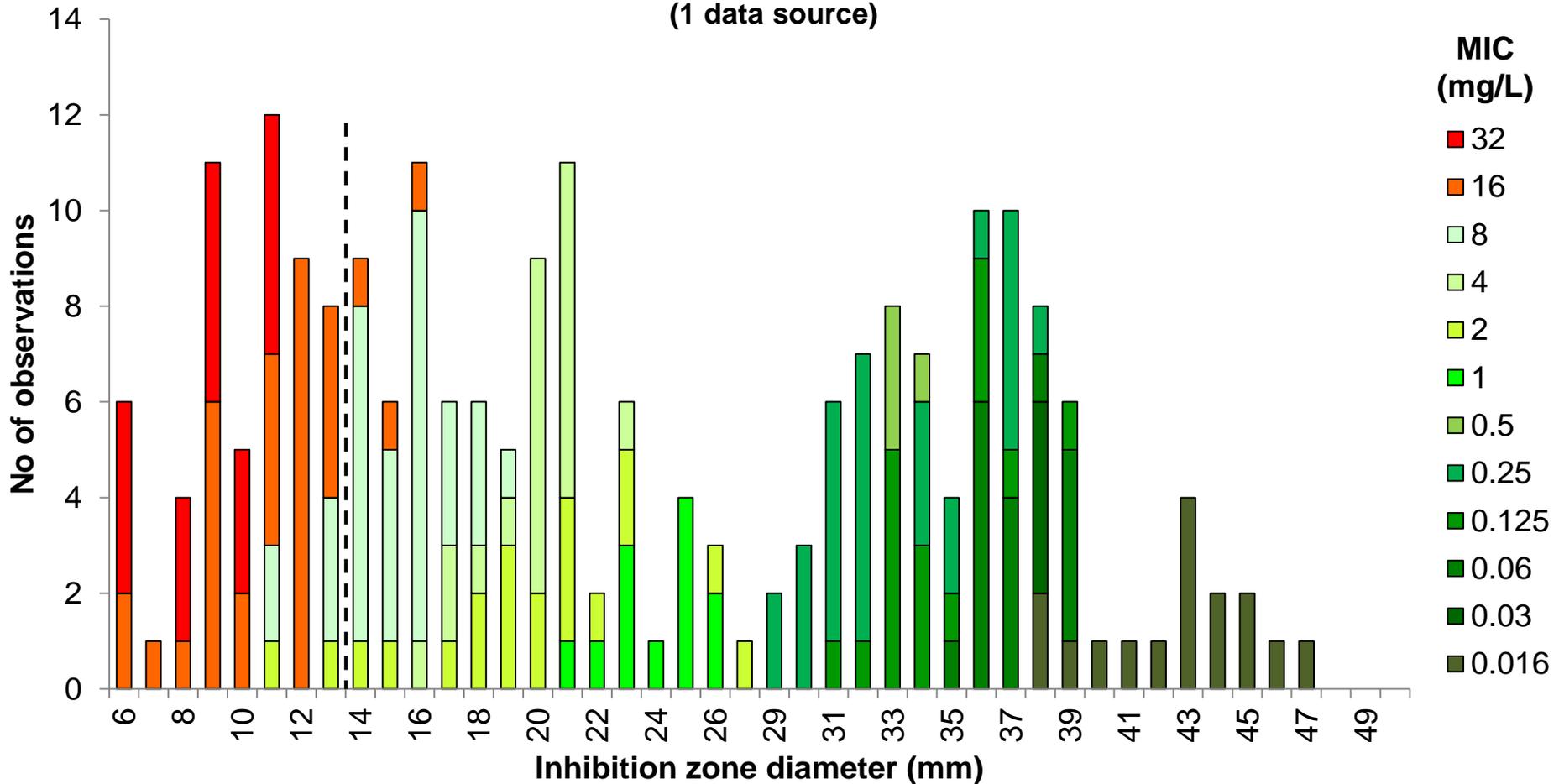
Meropenem-vaborbactam 20-10 µg vs. MIC *Enterobacterales*, 104 isolates (412 correlates)



Breakpoints	
MIC	S ≤ 8, R > 8 mg/L
Zone diameter	S ≥ 20, R < 20 mm

Meropenem-vaborbactam 20-10 µg vs. MIC *P. aeruginosa*, 55 isolates (220 correlates)

(1 data source)



Breakpoints

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

Zone diameter $S \geq 14$, $R < 14$ mm

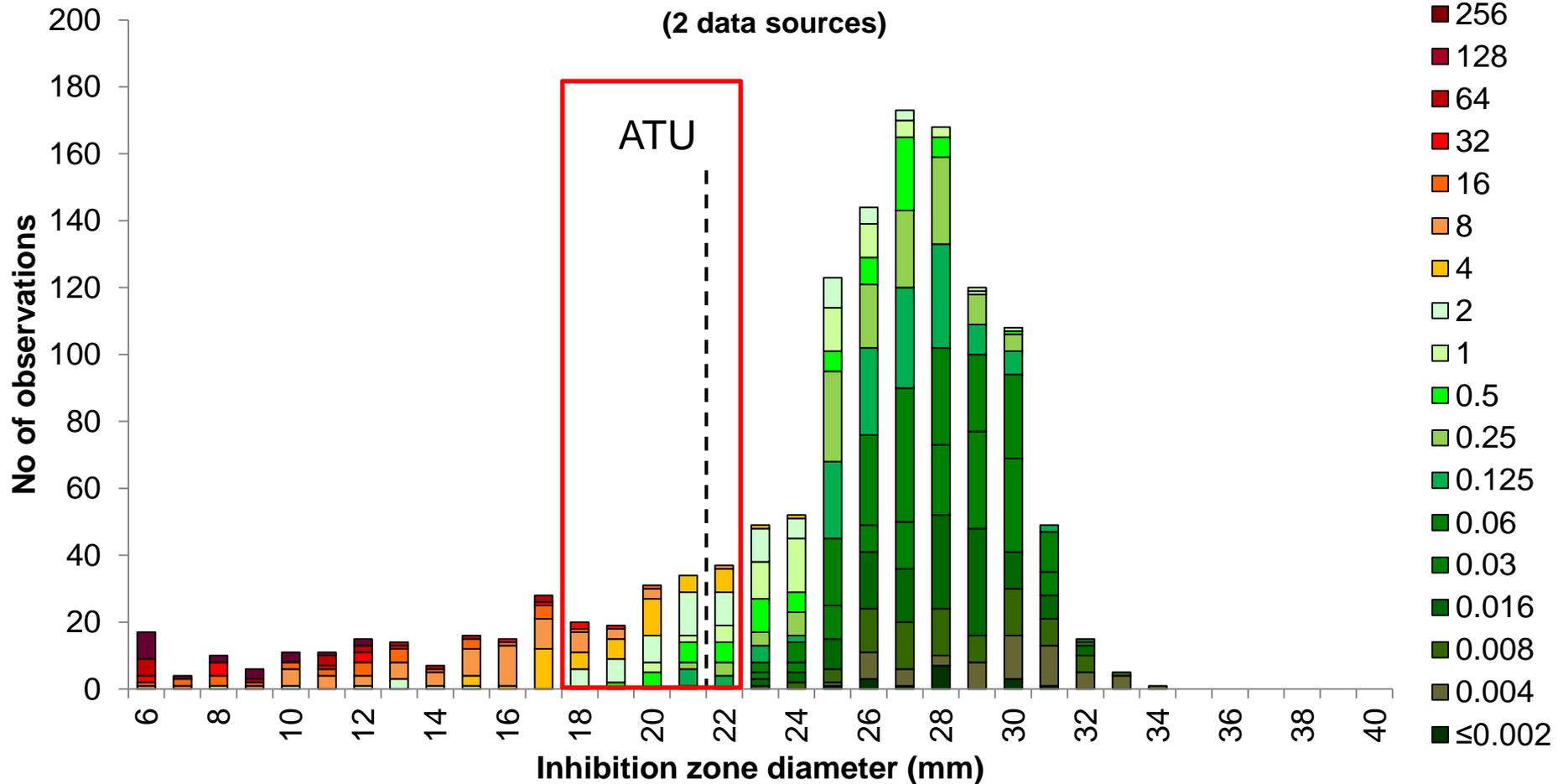
Cefiderocol AST

- Cefiderocol is a novel siderophore cephalosporin with a unique ability to enter the bacterial cells via the iron-transport system
- Cefiderocol has activity against Gram-negative bacteria, including ESBL-producing organisms and many carbapenemase producers
- Broth microdilution in iron-depleted Mueller-Hinton broth (ID-CAMHB)
 - See EUCAST Guidance document for MIC methodology and specific reading instructions for cefiderocol, <https://www.eucast.org/eucastguidancedocuments>
- Disk diffusion on standard un-supplemented Mueller-Hinton agar
 - Zone diameter breakpoints for *Enterobacterales* and *P. aeruginosa*
 - Zone diameter cut-off values corresponding to the PK-PD breakpoint for *Acinetobacter* spp. and *S. maltophilia*

Cefiderocol 30 µg vs. MIC

Enterobacterales, 382 isolates (1302 correlates)

(2 data sources)



Breakpoints

MIC

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

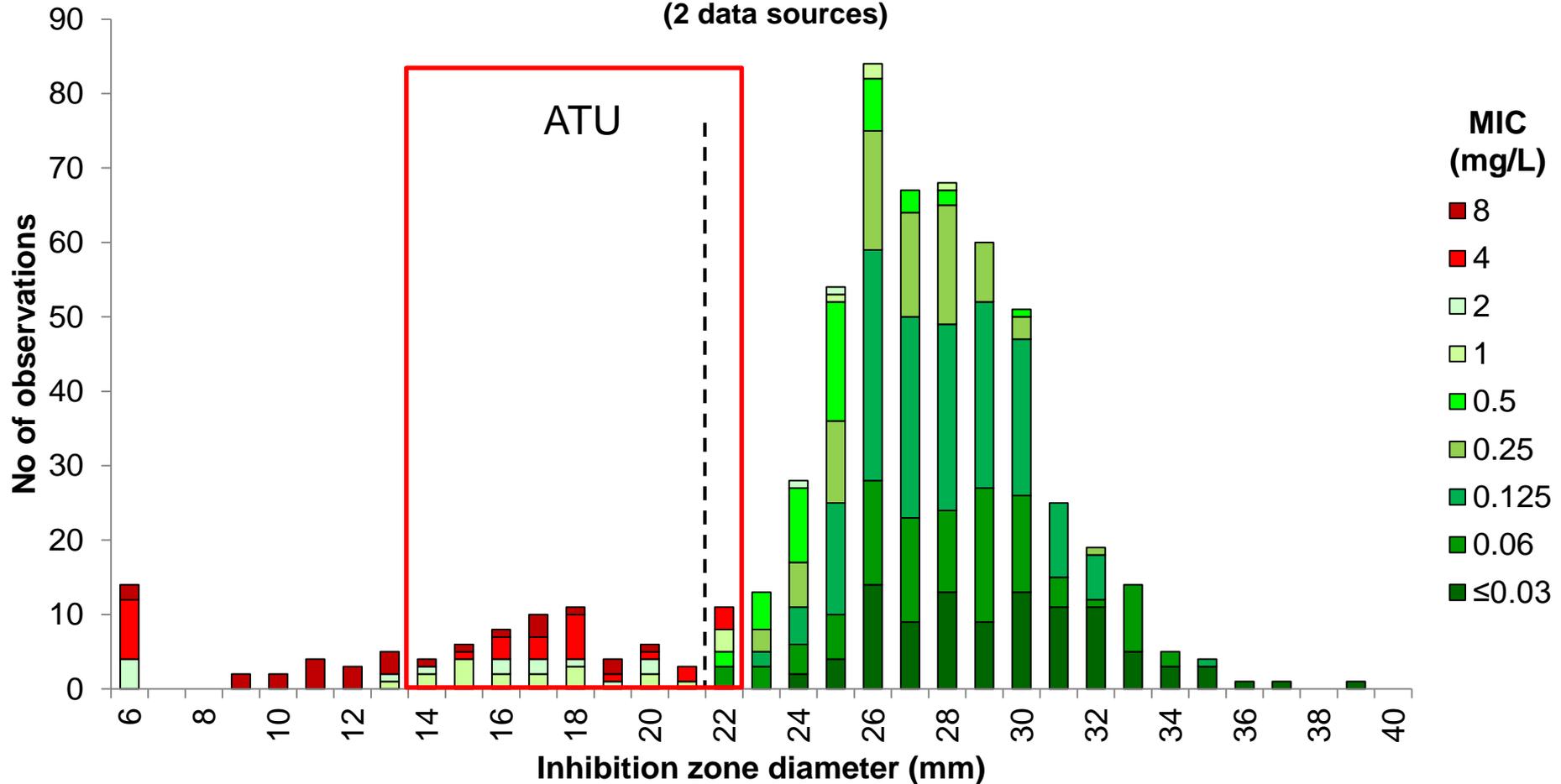
Zone diameter

$S \geq 22, R < 22$ mm

Cefiderocol 30 µg vs. MIC

P. aeruginosa, 152 isolates (588 correlates)

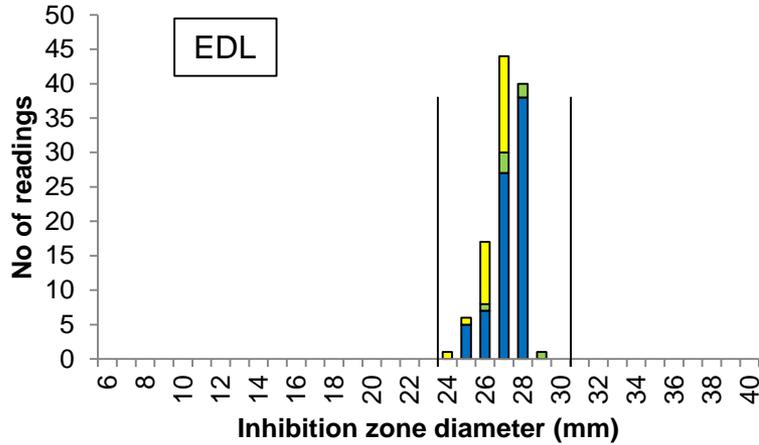
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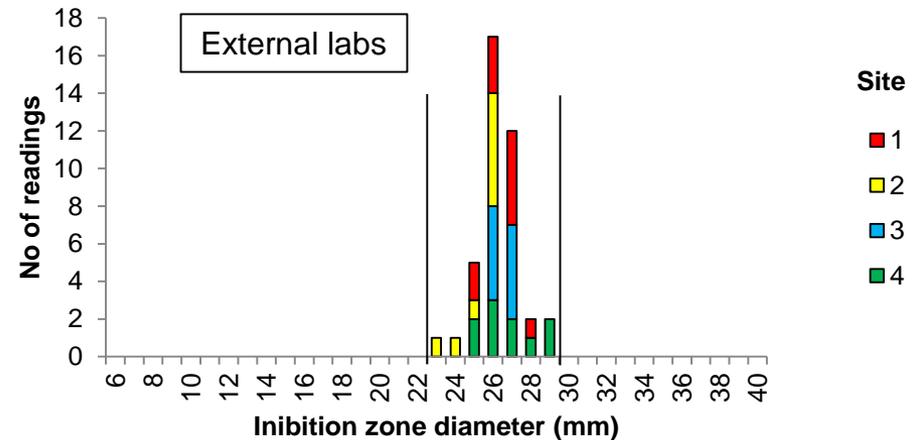
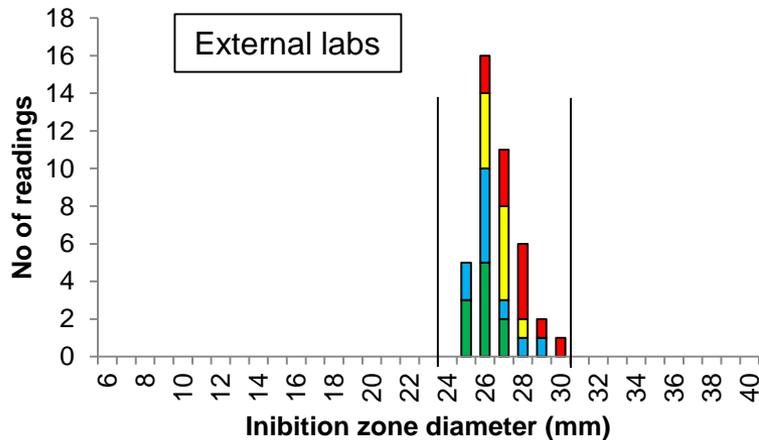
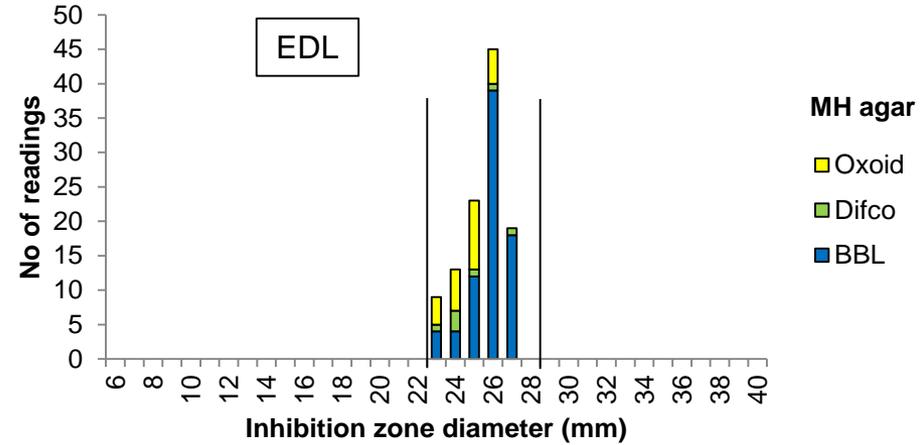
Breakpoints	
MIC	S ≤ 2, R > 2 mg/L
Zone diameter	S ≥ 22, R < 22 mm

Validation using QC strains

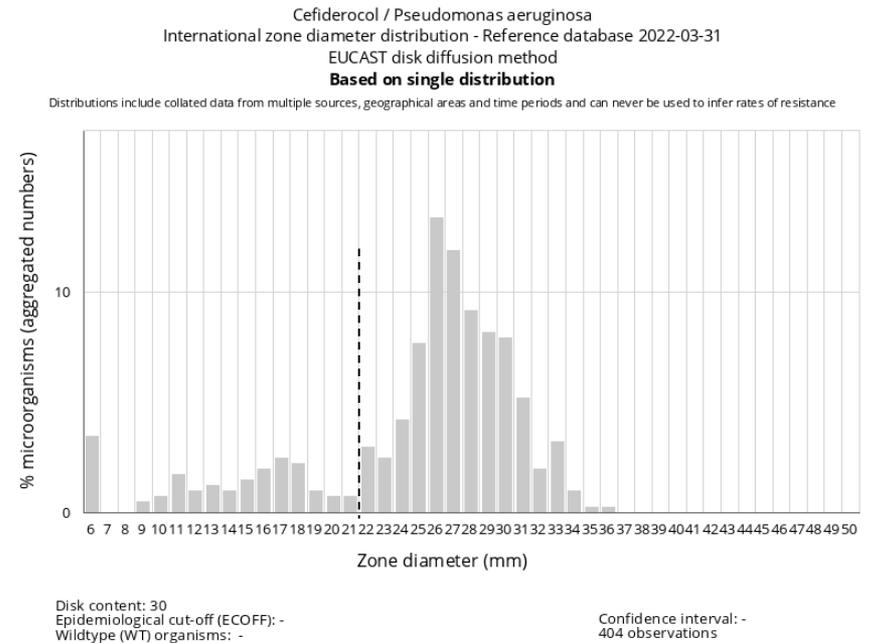
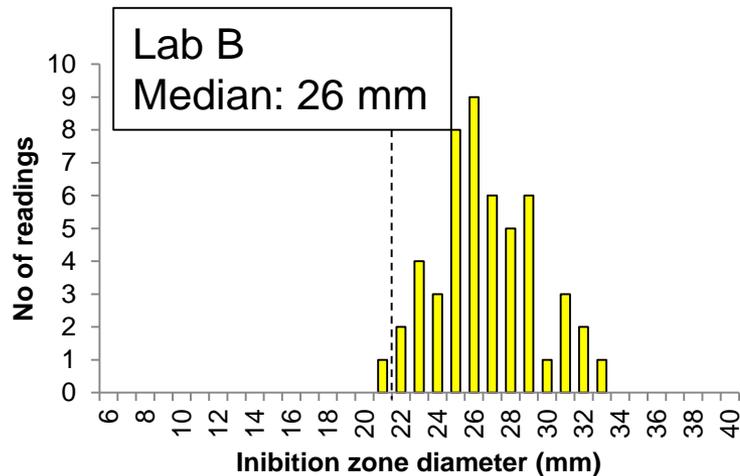
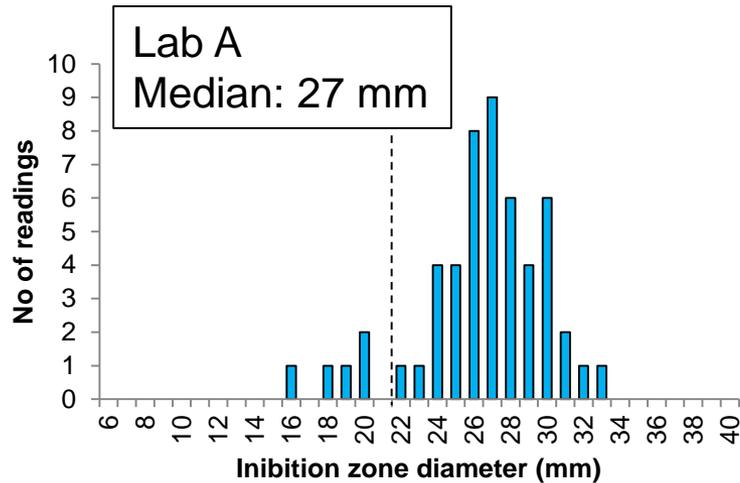
E. coli ATCC 25922



P. aeruginosa ATCC 27853



Validation using clinical isolates



EUCAST reference distribution
Median: 27-28 mm

MIC determination of cefiderocol

- In-house prepared plates with ID-CAMHB
- Commercial BMD panels (freeze-dried antibiotics)
 - Sensititre (Thermo Scientific)
 - Chelator in cefiderocol wells enables use of standard MH broth
 - Warning on too low MICs with current broth lots!
 - ComASP (Liofilchem)
 - ID-CAMHB
 - Specific reading instructions
- Gradient test
 - MIC Test Strip (Liofilchem)
 - For *P. aeruginosa* only!

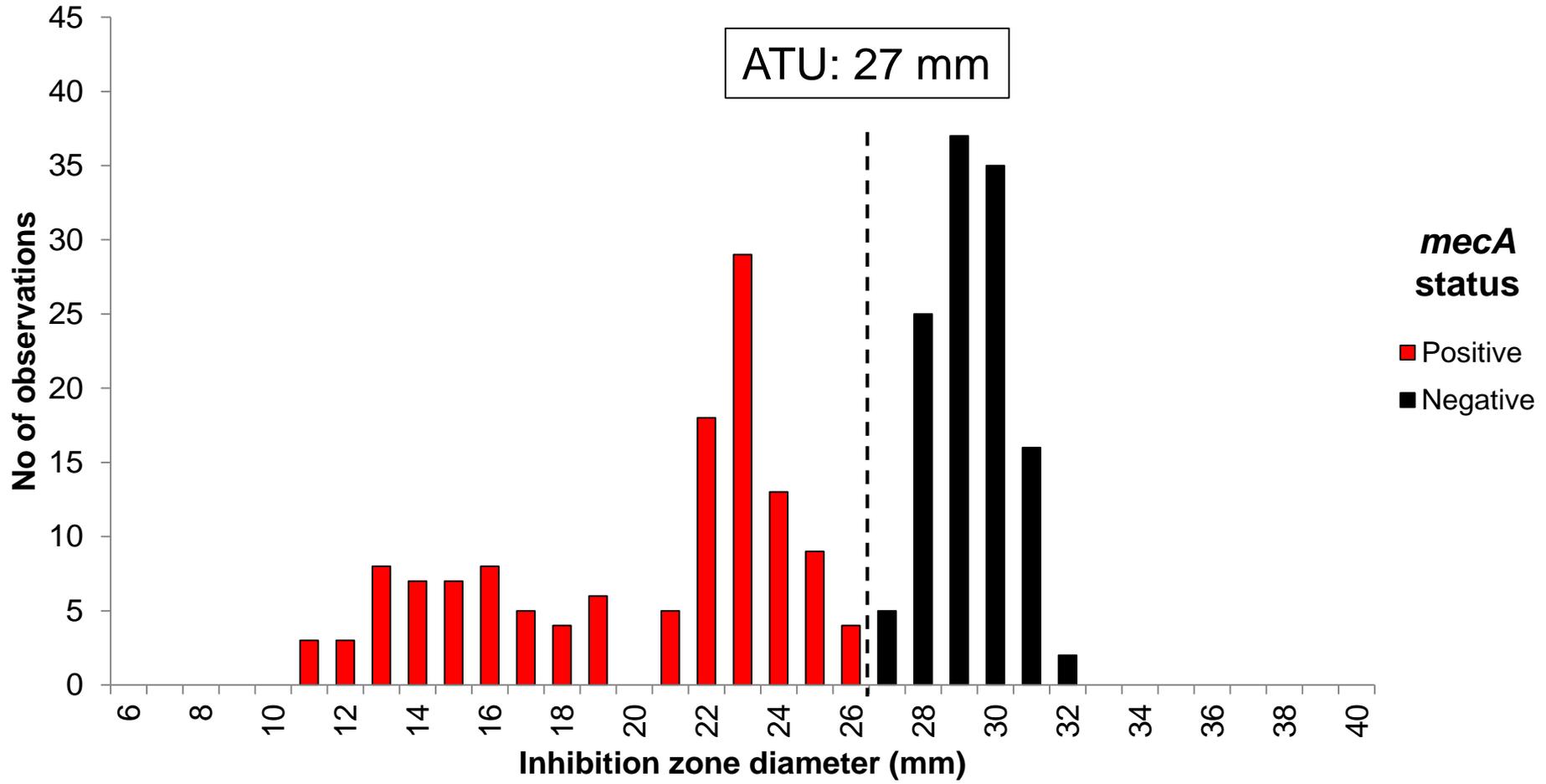
New screening breakpoints for methicillin resistance

EUCAST Breakpoint Tables v 12.0 *Staphylococcus* spp.

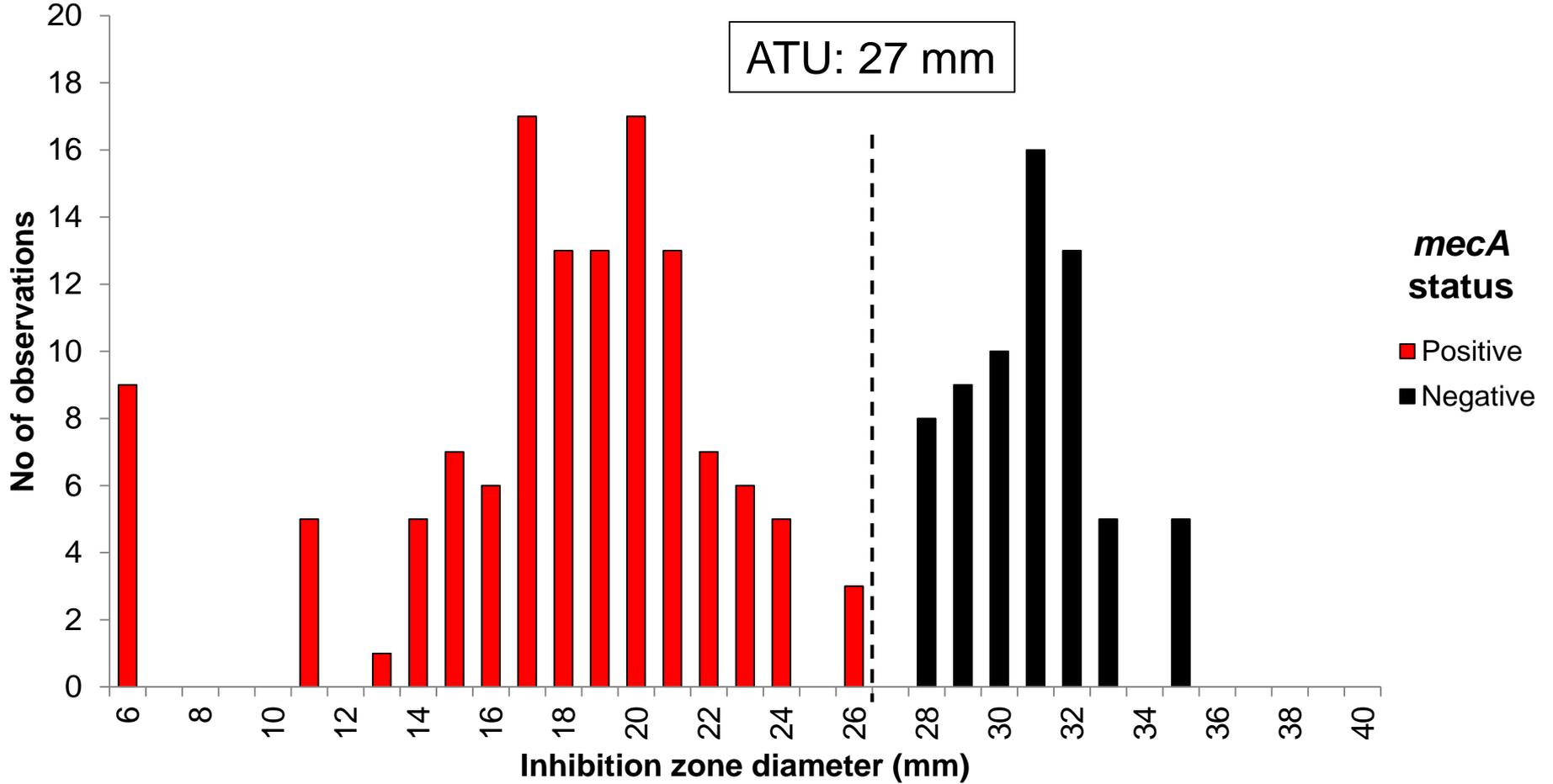
Cephalosporins	Disk content (µg)	Zone diameter breakpoints (mm)		
		S ≥	R <	ATU
Cefoxitin (screen only), <i>S. aureus</i> and coagulase-negative staphylococci except <i>S. epidermidis</i> and <i>S. lugdunensis</i>	30	22	22	
Cefoxitin (screen only), <i>S. epidermidis</i> and <i>S. lugdunensis</i>	30	27	27	27
Cefoxitin (screen only), <i>S. pseudintermedius</i> , <i>S. schleiferi</i> and <i>S. coagulans</i>		Note ^C	Note ^C	

C. In *S. pseudintermedius*, *S. schleiferi* and *S. coagulans* the cefoxitin disk is less predictive for the detection of methicillin resistance than in other staphylococci. Use the oxacillin 1 µg disk with zone diameter breakpoints S≥20, R<20 mm.

**Cefoxitin 30 μ g vs. *mecA* status
S. lugdunensis, 43 isolates (249 correlates)**



**Cefoxitin 30 μ g vs. *mecA* status
S. epidermidis, 100 isolates (193 correlates)**



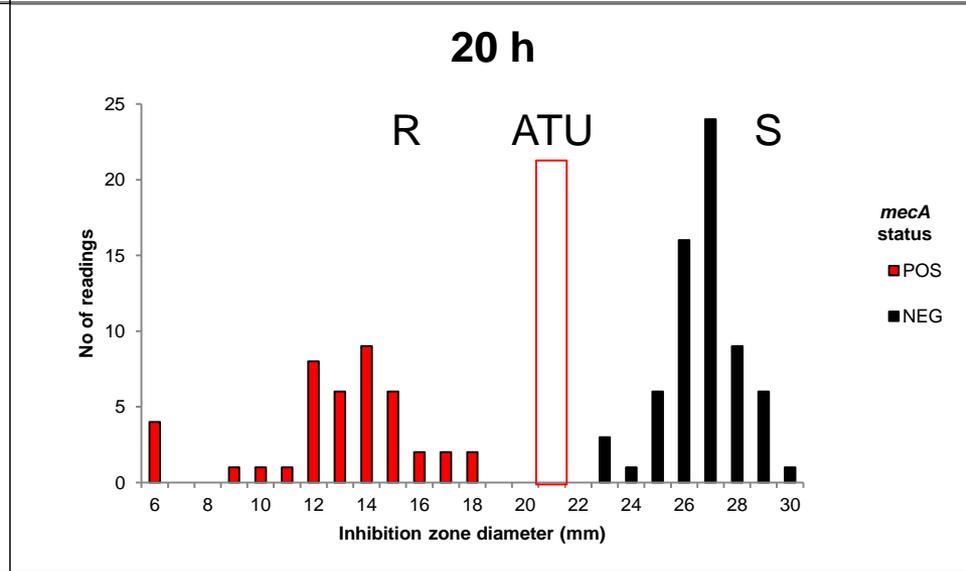
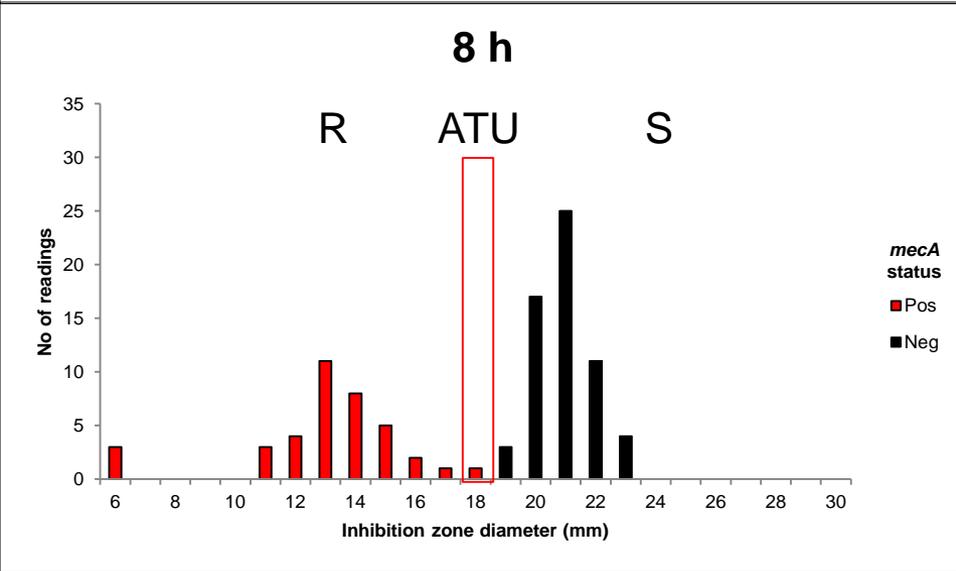
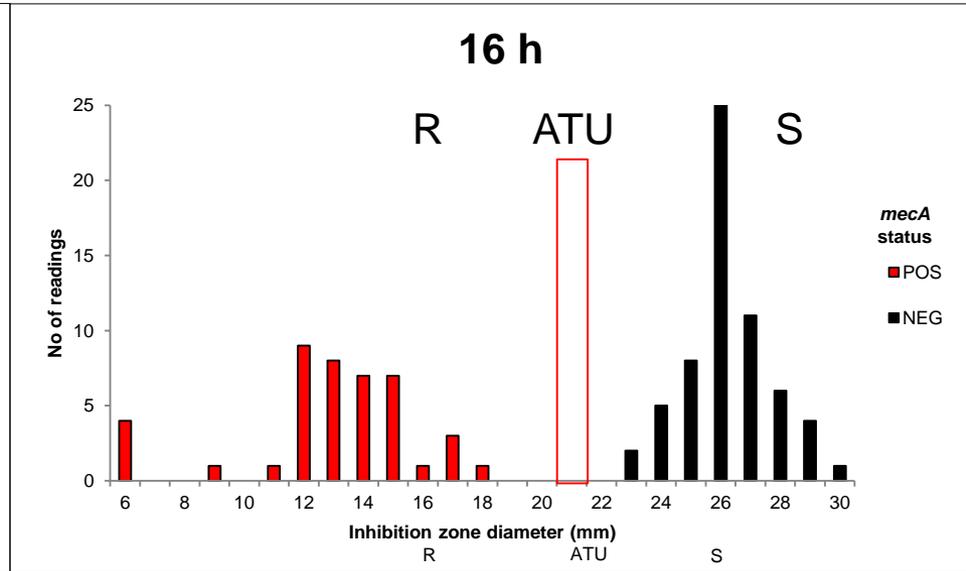
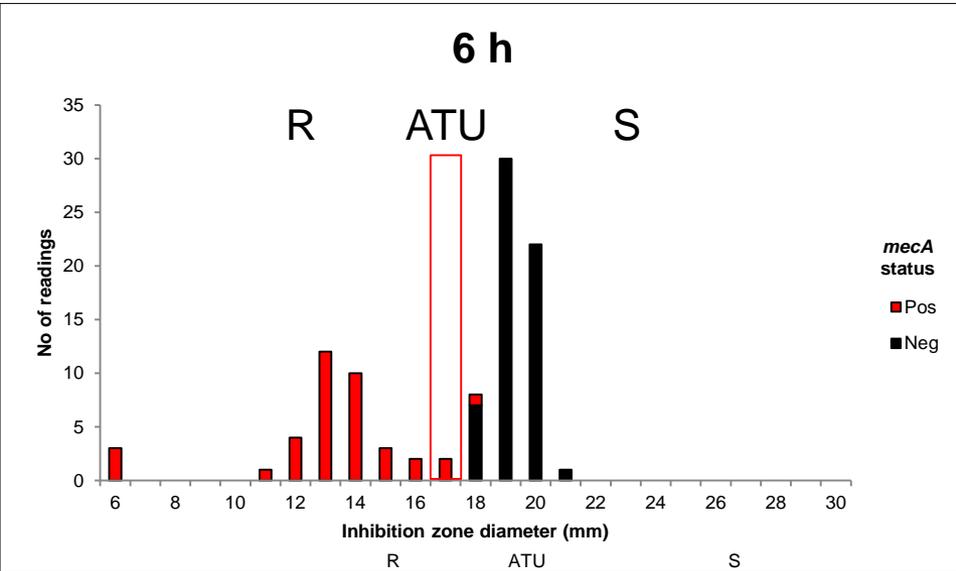
New RAST criteria

- EUCAST RAST: Rapid susceptibility test results directly from positive blood cultures
 - Readings at 4, 6 and/or 8 hours
- New from April 2022: RAST breakpoints for 16-20 h incubation
 - Developed for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae*
 - To be used only when it is not possible to read results after 4, 6 and/or 8 hours incubation
 - Information on testing, implementation and reading of zones are available in the RAST manual, QC table and Breakpoint tables

https://www.eucast.org/rapid_ast_in_blood_cultures

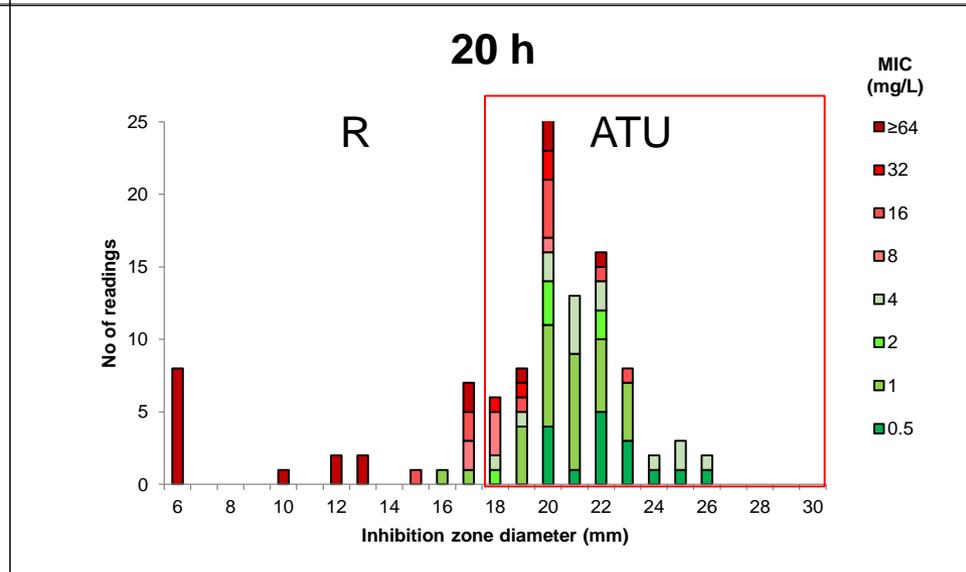
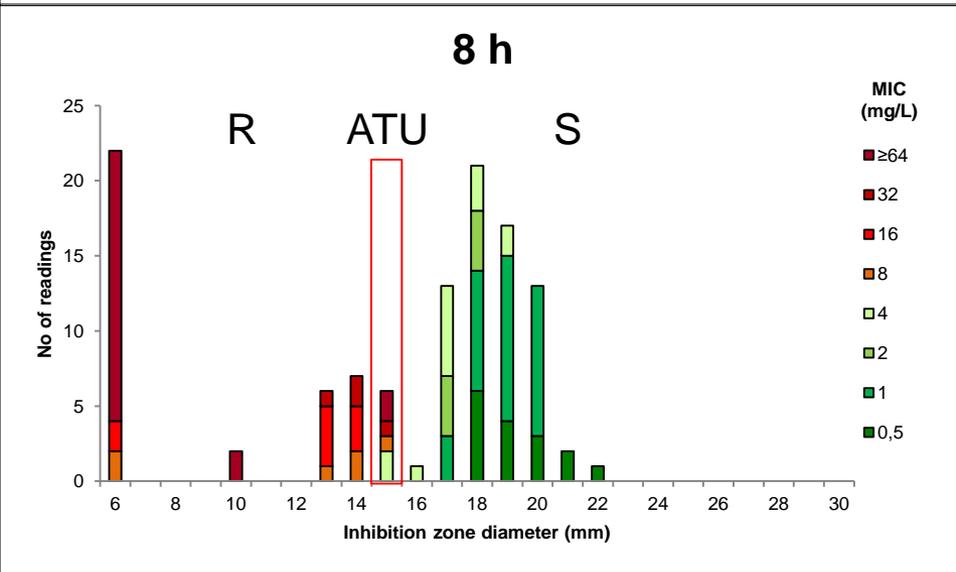
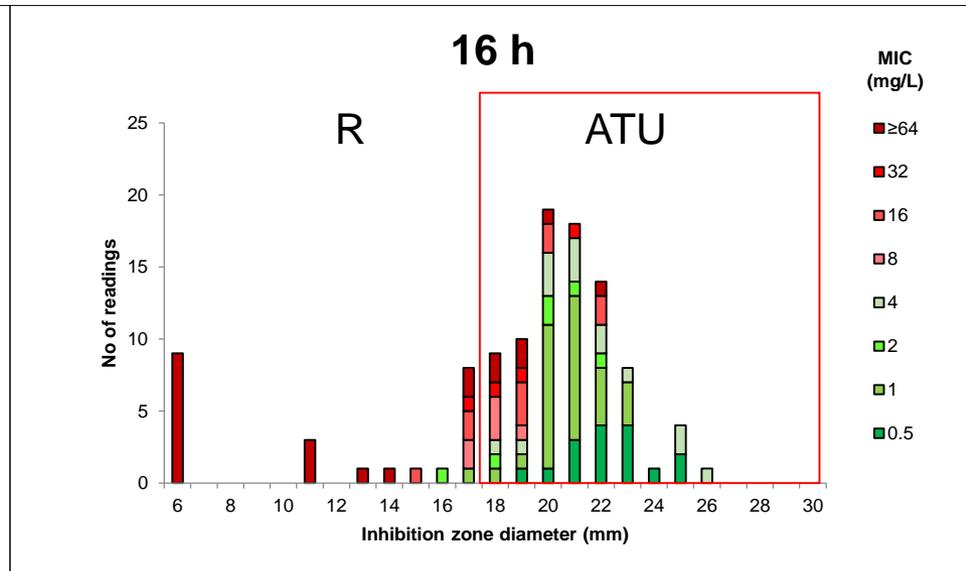
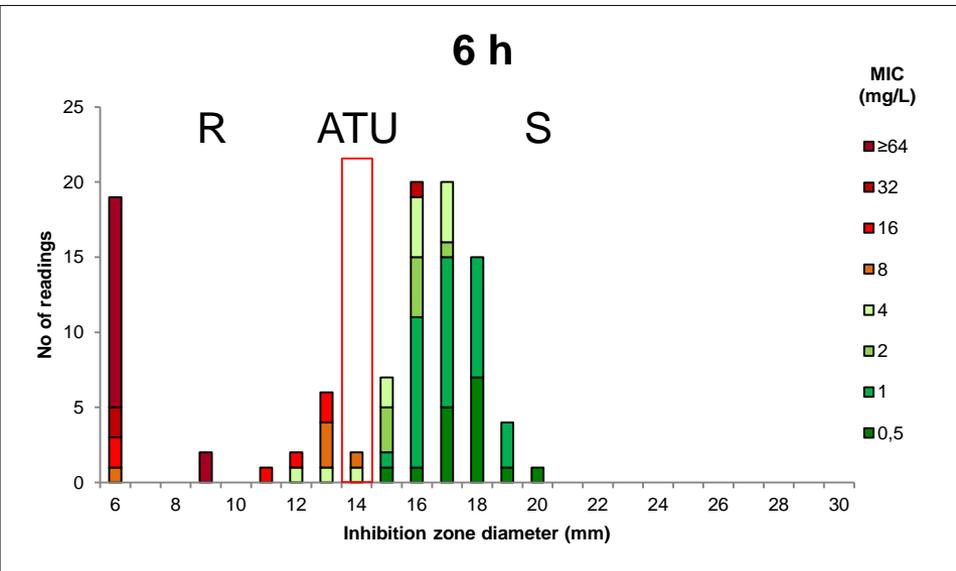
S. aureus and ceftiofur

Better separation after 16-20 h



P. aeruginosa and ceftolozane-tazobactam

Poorer separation after 16-20 h



Organisms added to the breakpoint table the last 5 years

Organism	Year
<i>Aerococcus sanguincola</i> and <i>urinae</i>	2017
<i>Kingella kingae</i>	2017
<i>Aeromonas</i> spp.	2018
<i>Burkholderia pseudomallei</i>	2020
<i>Achromobacter xylosoxidans</i>	2021
<i>Bacillus</i> spp.	2021
<i>Vibrio</i> spp.	2022
Anaerobic bacteria (selected species)	2022

On-going:

Corynebacterium diphtheriae and *ulcerans*

Nocardia spp.

Vibrio spp. methodology

- Initial testing of 10 QC strains of different *Vibrio* spp. confirmed the use of EUCAST standard methodology for non-fastidious organisms.
 - Un-supplemented MH, McF 0.5, inc in air at 35°C for 16-20 h.
- Testing was performed for 12 antimicrobial agents:
 - MIC determination with broth microdilution (BMD) according to ISO 20776-1.
 - Custom-made Sensititre panels
 - Disk diffusion (DD) according to EUCAST
 - MH agar from two manufacturers (BBL and Oxoid)

Vibrio spp. isolates

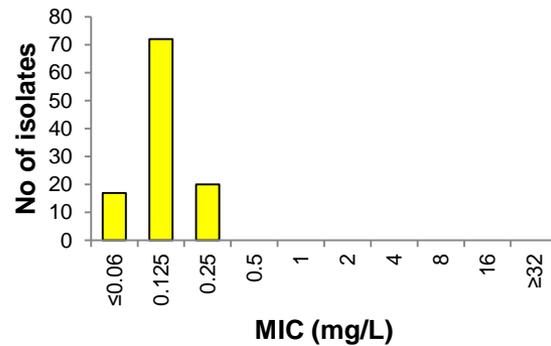
- *Vibrio* spp. isolates were collected from different countries.
 - Sweden, Norway, Finland, Spain, UK, India, USA
- 5 clinically most important species:

Organism	No
<i>Vibrio alginolyticus</i>	79
<i>Vibrio cholerae</i>	87
<i>Vibrio fluvialis</i>	53
<i>Vibrio parahaemolyticus</i>	77
<i>Vibrio vulnificus</i>	75
TOTAL	371

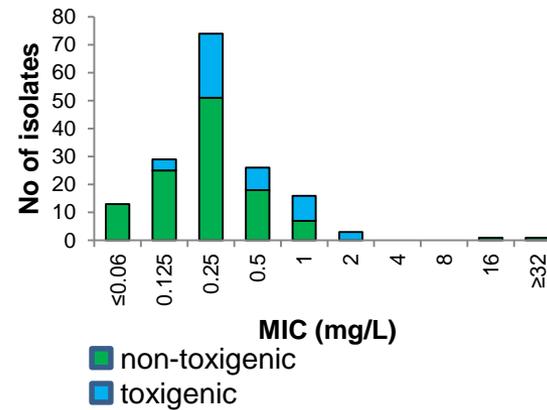
- Additionally 50 toxin-producing *V. cholerae* were tested with BMD at CDC, USA.

Piperacillin-tazobactam MIC

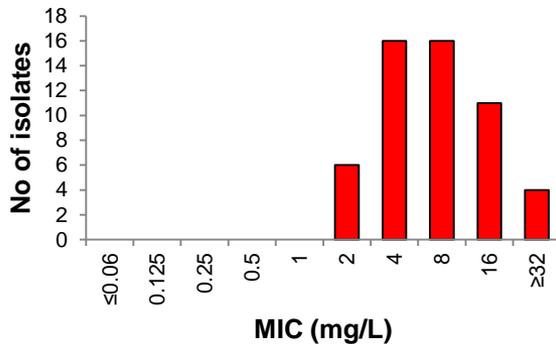
V. alginolyticus



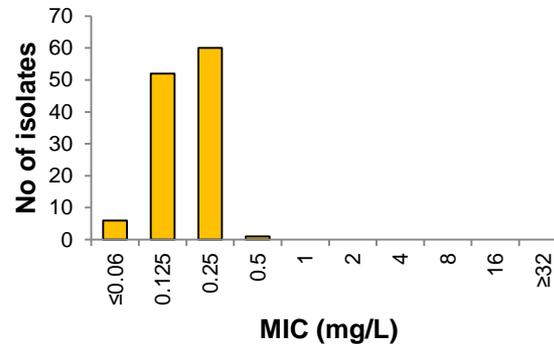
V. cholerae



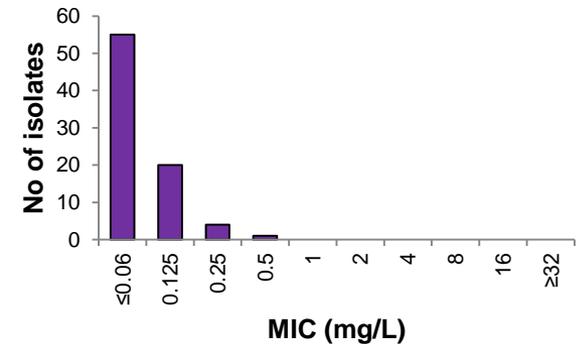
V. fluvialis



V. parahaemolyticus

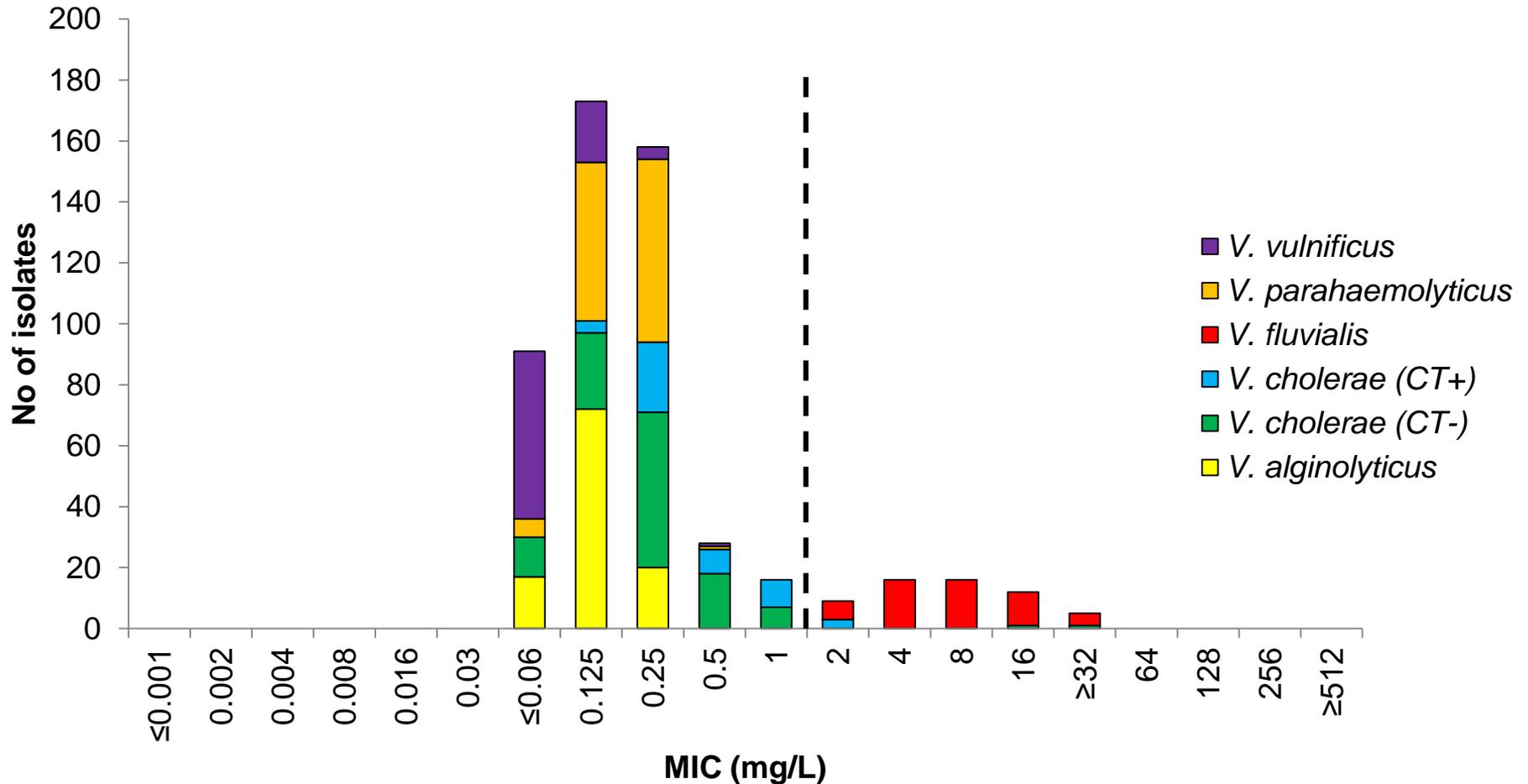


V. vulnificus



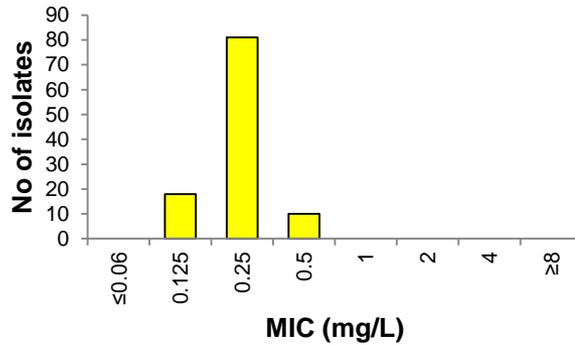
Piperacillin-tazobactam MIC

Piperacillin-tazobactam MIC distribution *Vibrio* spp.

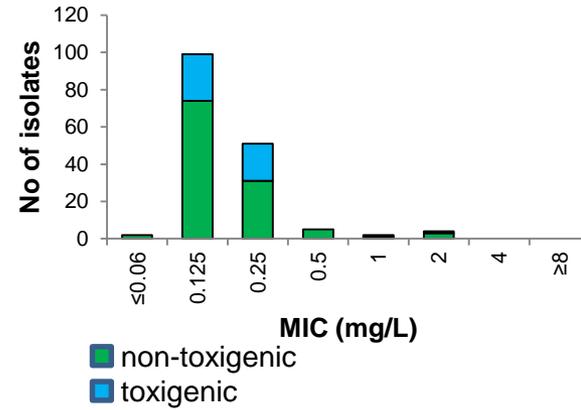


Doxycycline MIC

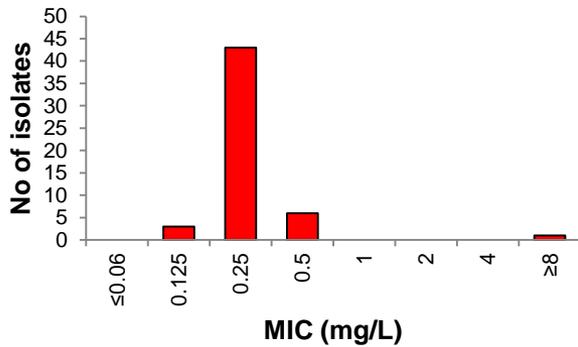
V. alginolyticus



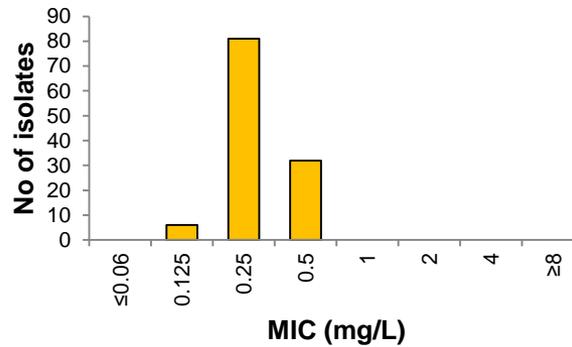
V. cholerae



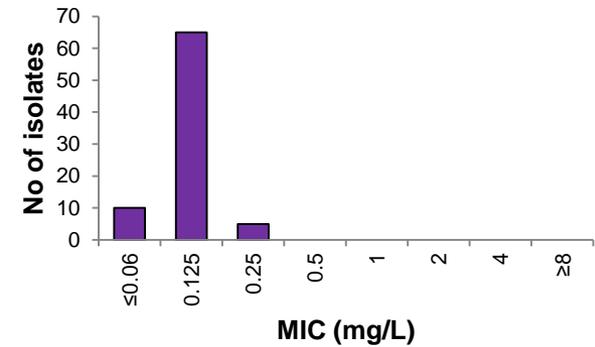
V. fluvialis



V. parahaemolyticus

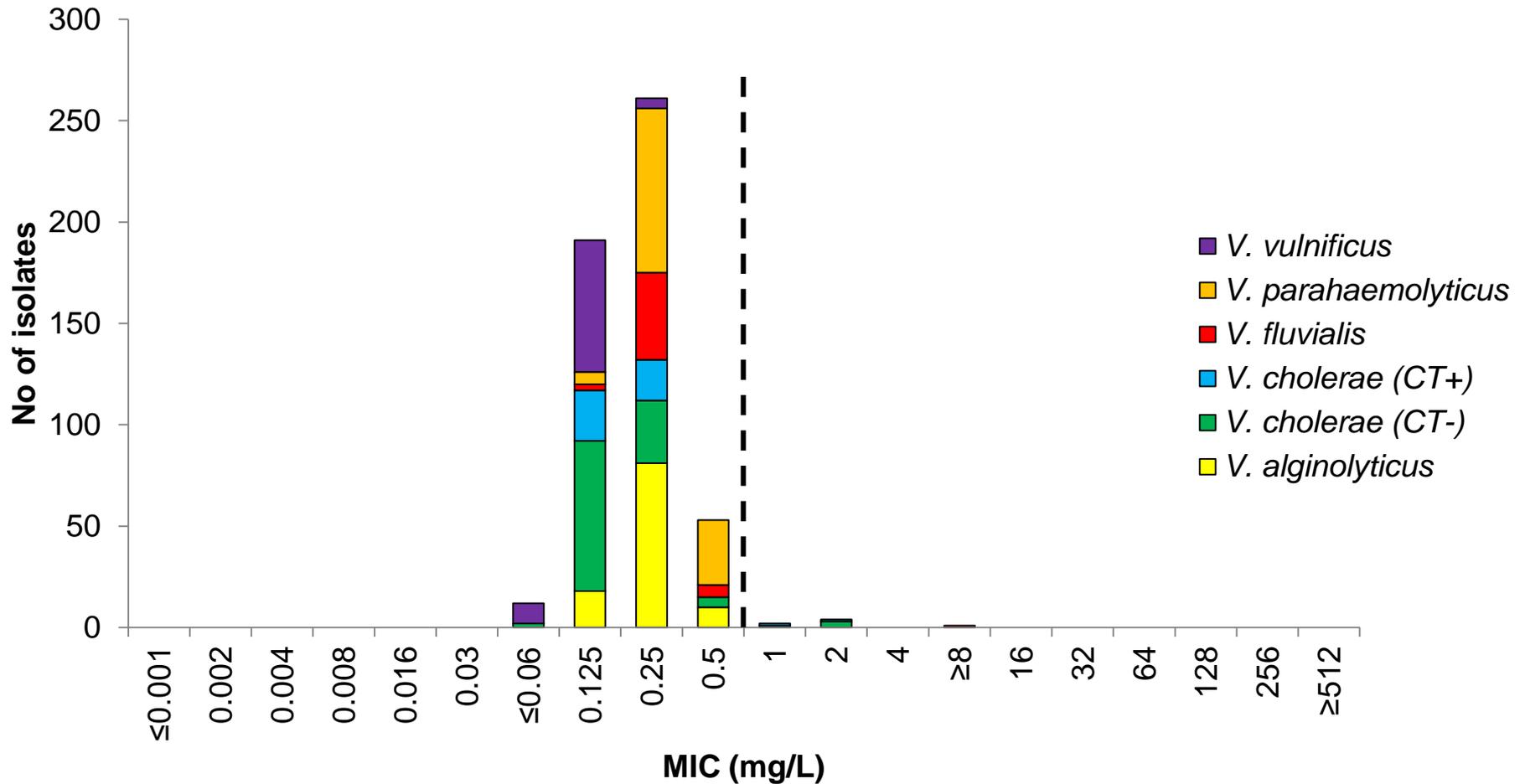


V. vulnificus



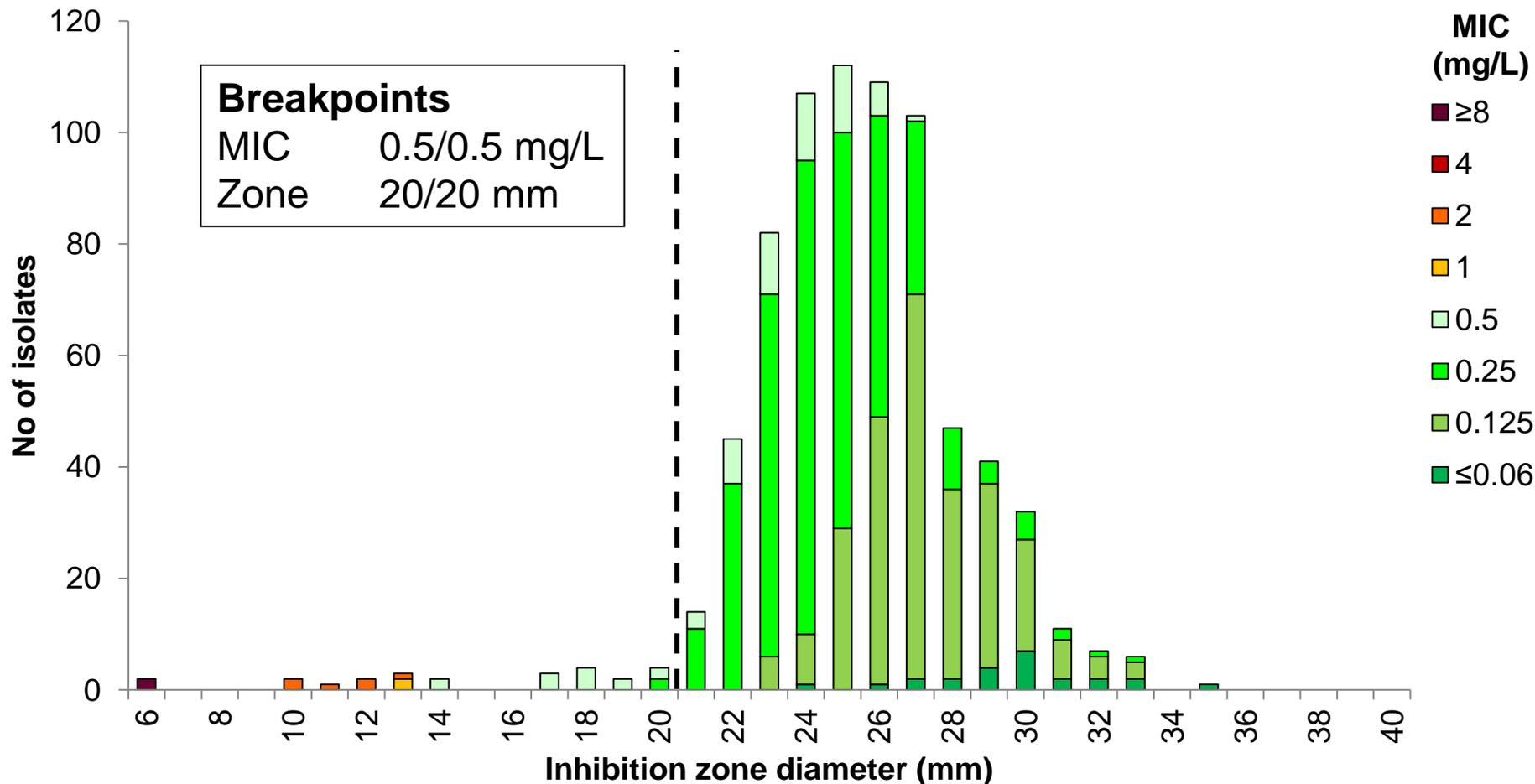
Doxycycline MIC

Doxycycline MIC distribution *Vibrio* spp.



Tetracycline vs. doxycycline

Tetracycline 30 µg vs. Doxycycline MIC
Vibrio spp., 371 isolates



Breakpoints for *Vibrio* spp.

Breakpoints are valid for *V. alginolyticus*, *V. cholerae*, *V. fluvialis*, *V. parahaemolyticus* and *V. vulnificus*.

Antimicrobial agent	MIC breakpoints		Disk content	Zone diameter	
	S ≤	R >		S ≥	R <
Piperacillin-tazobactam	1	1	30-6	26	26
Cefotaxime	0.25	0.25	5	21	21
Cefotaxime, <i>V. fluvialis</i>	IE	IE		IE	IE
Ceftazidime	1	1	10	22	22
Meropenem	0.5	0.5	10	24	24
Ciprofloxacin	0.25	0.25	5	23	23
Levofloxacin	0.25	0.25	5	23	23
Pefloxacin (screen only)	NA	NA	5	20	20
Azithromycin	4	4	15	16	16
Erythromycin (screen only)	NA	NA	15	12	12
Doxycycline	0.5	0.5		Note	Note
Tetracycline (screen only)	NA	NA	30	20	20
Trimethoprim-sulfamethoxazole	0.5	0.5	1.25-23.75	18	18

Corynebacterium spp.

- Breakpoints for *Corynebacterium* spp. available since 2014.
 - Developed for species other than *C. diphtheriae* and *C. ulcerans*.
- Participating laboratories
 - National Reference Center for Diphtheria, Institut Pasteur, France
 - Bavarian Food and Health Authority (LGL), Germany
- Study isolates
 - 100 *C. diphtheriae* per site
 - 100 *C. ulcerans* per site

C. diphtheriae and *C. ulcerans*

Antimicrobial agent	BMD panel range (mg/L)	Disk potency (µg)
Benzylopenicillin	0.015-2	1 unit
Ampicillin	-	2
Amoxicillin	0.015-2	-
Cefotaxime	0.015-2	5
Meropenem	0.015-2	10
Ciprofloxacin	0.03-4	5
Erythromycin	0.008-2	15
Clindamycin	0.03-8	2
Doxycycline	0.03-2	-
Tetracycline	0.06-4	30
Linezolid	0.06-4	10
Rifampicin	0.008-2	5
Trimethoprim-sulfamethoxazole	0.12-8	1.25-23.75

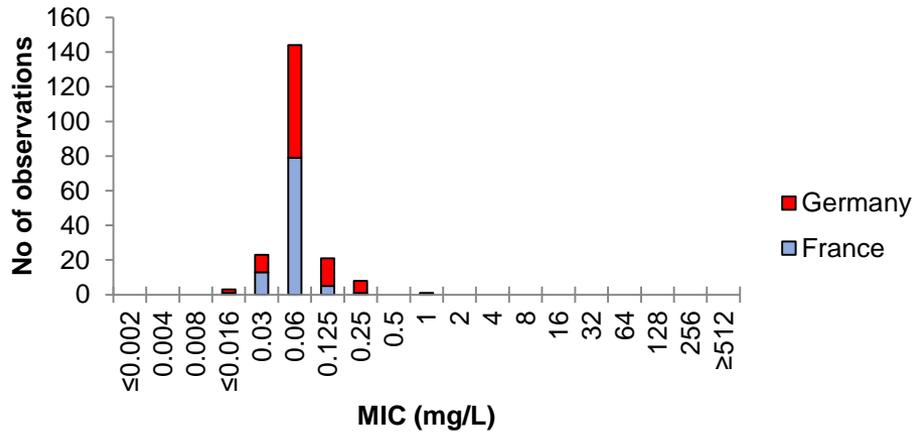
C. diphtheriae and *C. ulcerans*

- Disk diffusion and broth microdilution in parallel
 - MH-F agar, McF 0.5, inc in 5% CO₂, 35°C for 16-20 h
- All materials quality controlled by the EDL
 - Sensititre custom-made panels
 - MH-F broth
 - MH-F agar (local)
- Initial validation study:
 - 8 clinical isolates of *Corynebacterium* spp.
 - QC: *S. pneumoniae* ATCC 49619

Meropenem

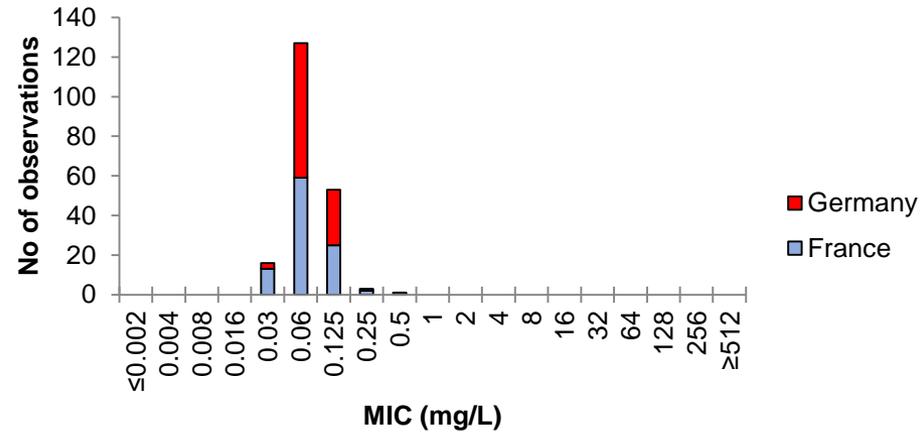
C. diphtheriae

Meropenem
C. diphtheriae, 200 isolates

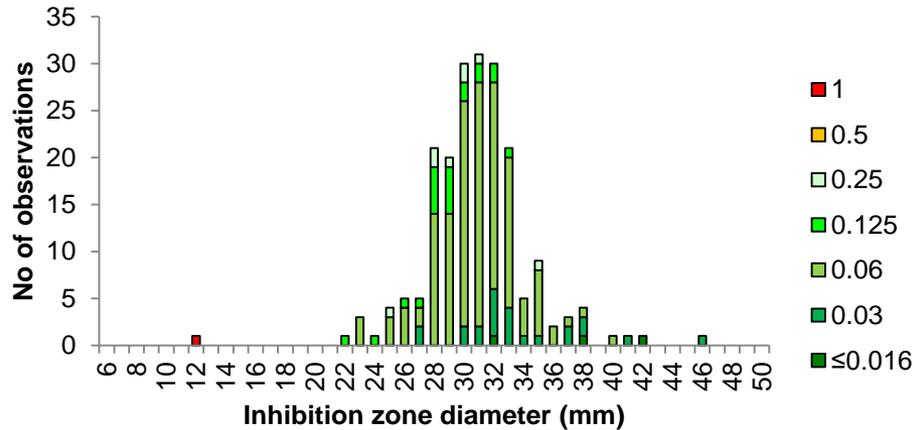


C. ulcerans

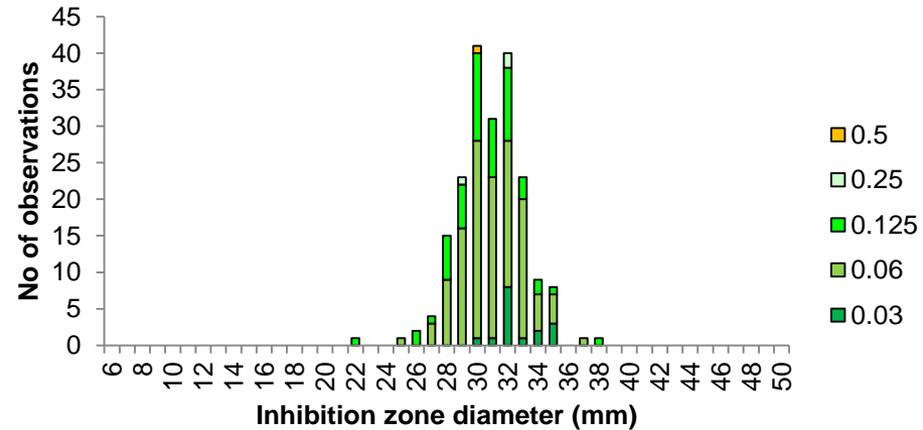
Meropenem
C. ulcerans, 200 isolates



Meropenem 10 µg vs. MIC
C. diphtheriae, 200 isolates



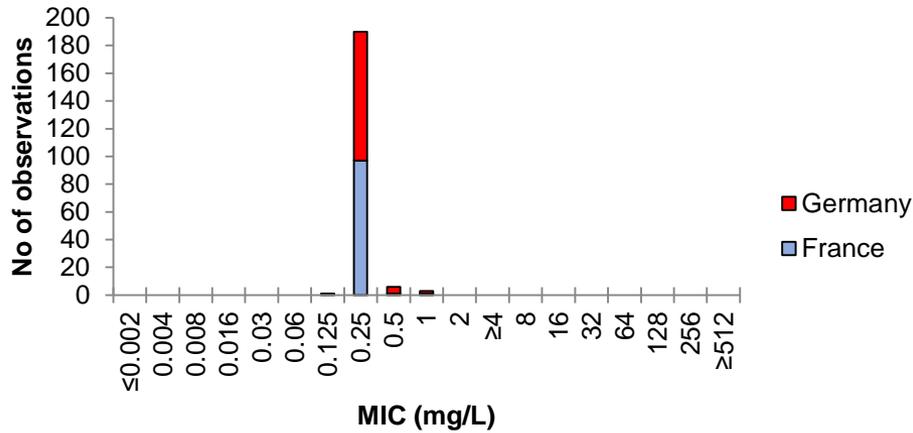
Meropenem 10 µg vs. MIC
C. ulcerans, 200 isolates



Linezolid

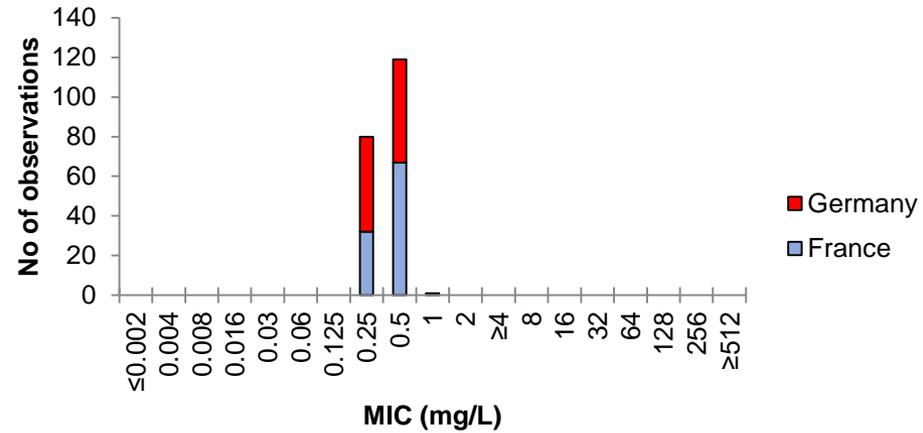
C. diphtheriae

Linezolid
C. diphtheriae, 200 isolates

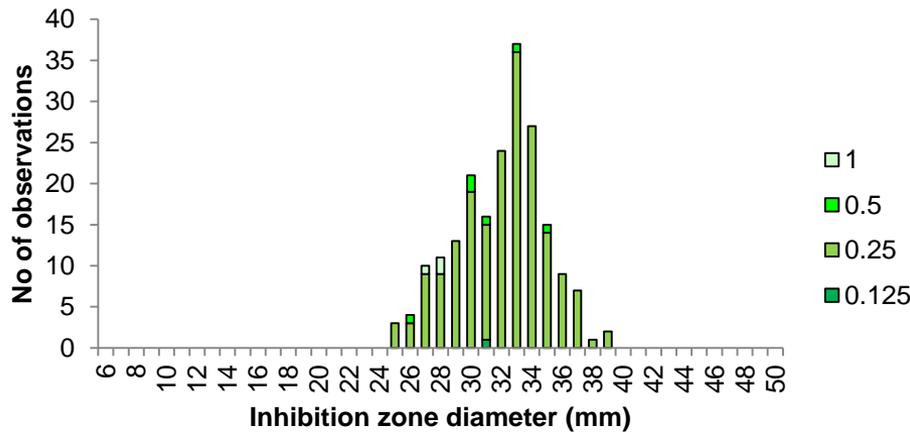


C. ulcerans

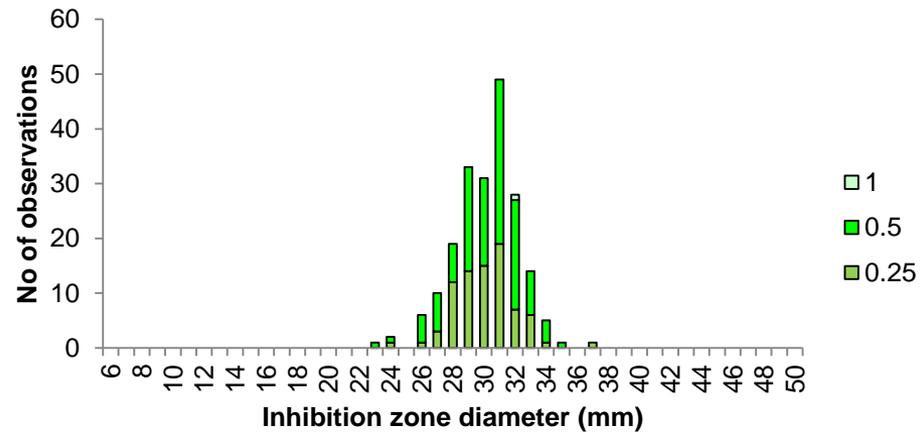
Linezolid
C. ulcerans, 200 isolates



Linezolid 10 µg vs. MIC
C. diphtheriae, 200 isolates

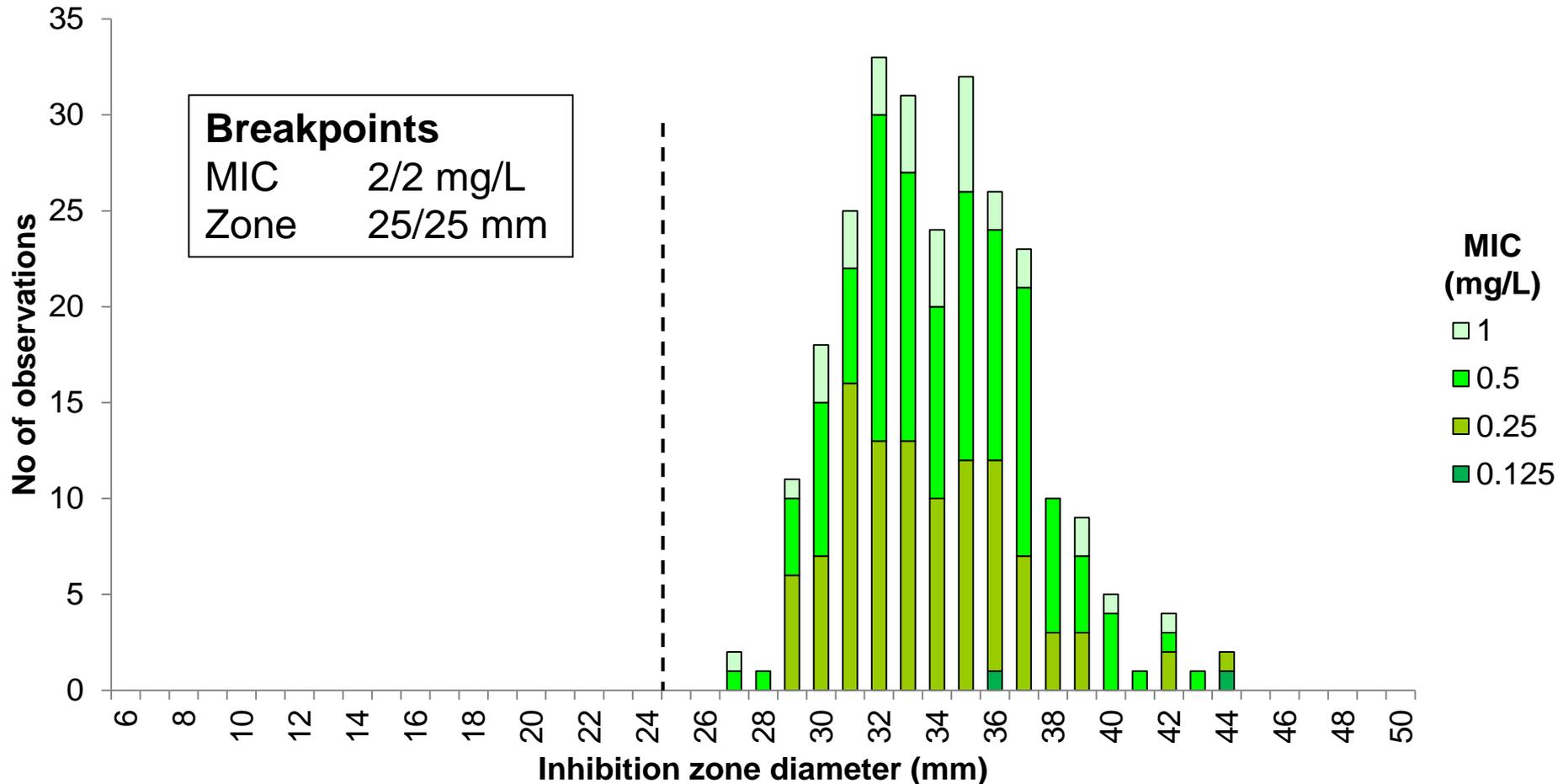


Linezolid 10 µg vs. MIC
C. ulcerans, 200 isolates



Linezolid

Linezolid 10 µg vs. MIC
Other *Corynebacterium* spp., 258 isolates



Reference BMD testing

- Enterobacterales
- Pseudomonas / Acinetobacter
- Gram-negative extended
 - Including cefiderocol, imipenem-relebactam, meropenem-vaborbactam
- Staphylococci / Enterococci
 - Including dalbavancin
- Streptococci
- Haemophilus
- Agar dilution Fosfomycin (0.25-256 mg/L)

More information on <http://mikrobiologi.org/referenslaboratorium>

Thanks!

- The EDL staff: Amra Basic, Onur Karatuna, Lucia Ortega, Lized Rodriguez and Gunnar Kahlmeter
- Emma Jonasson, Clinical Microbiology Växjö (RAST)
- **All clinical laboratories participating in studies, and contributing with data and/or isolates**

Questions and/or problem?

Contact us!

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