



What is going on in the EUCAST Development Laboratory (EDL)?

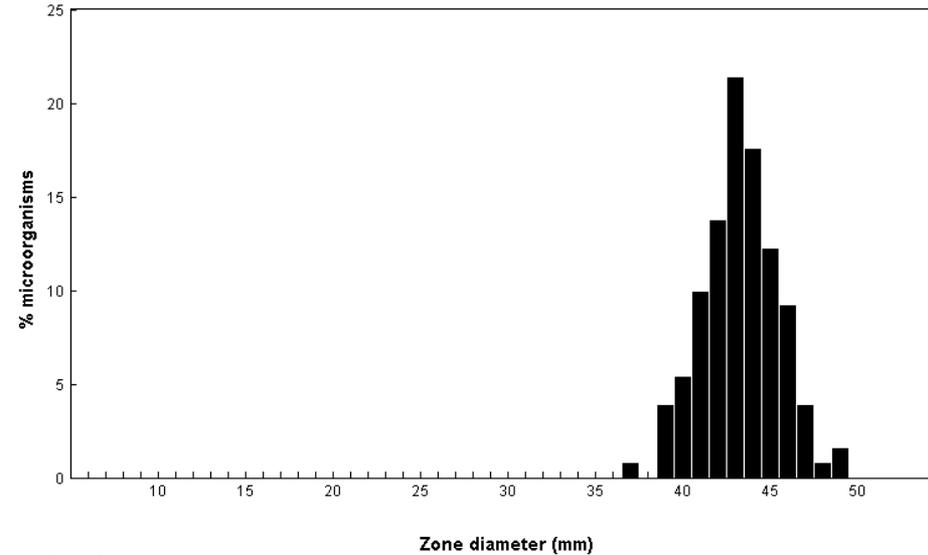
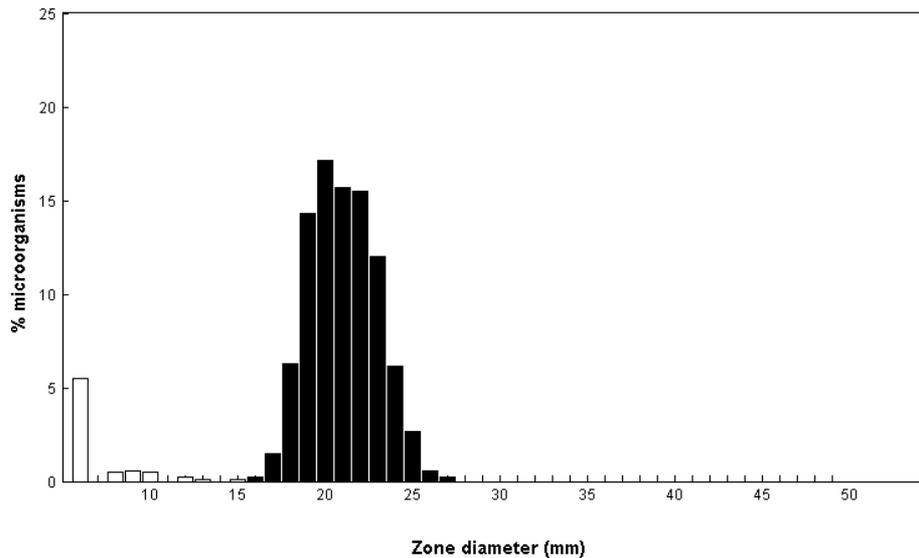
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EUCAST Development Laboratory

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.
- 18 agents ongoing
 - Data analysis of previously produced data
 - Disk potency studies
 - QC criteria
 - MIC vs. zone diameter correlates to establish zone diameter breakpoints

Selecting disk potency

- Optimized inhibition zone size

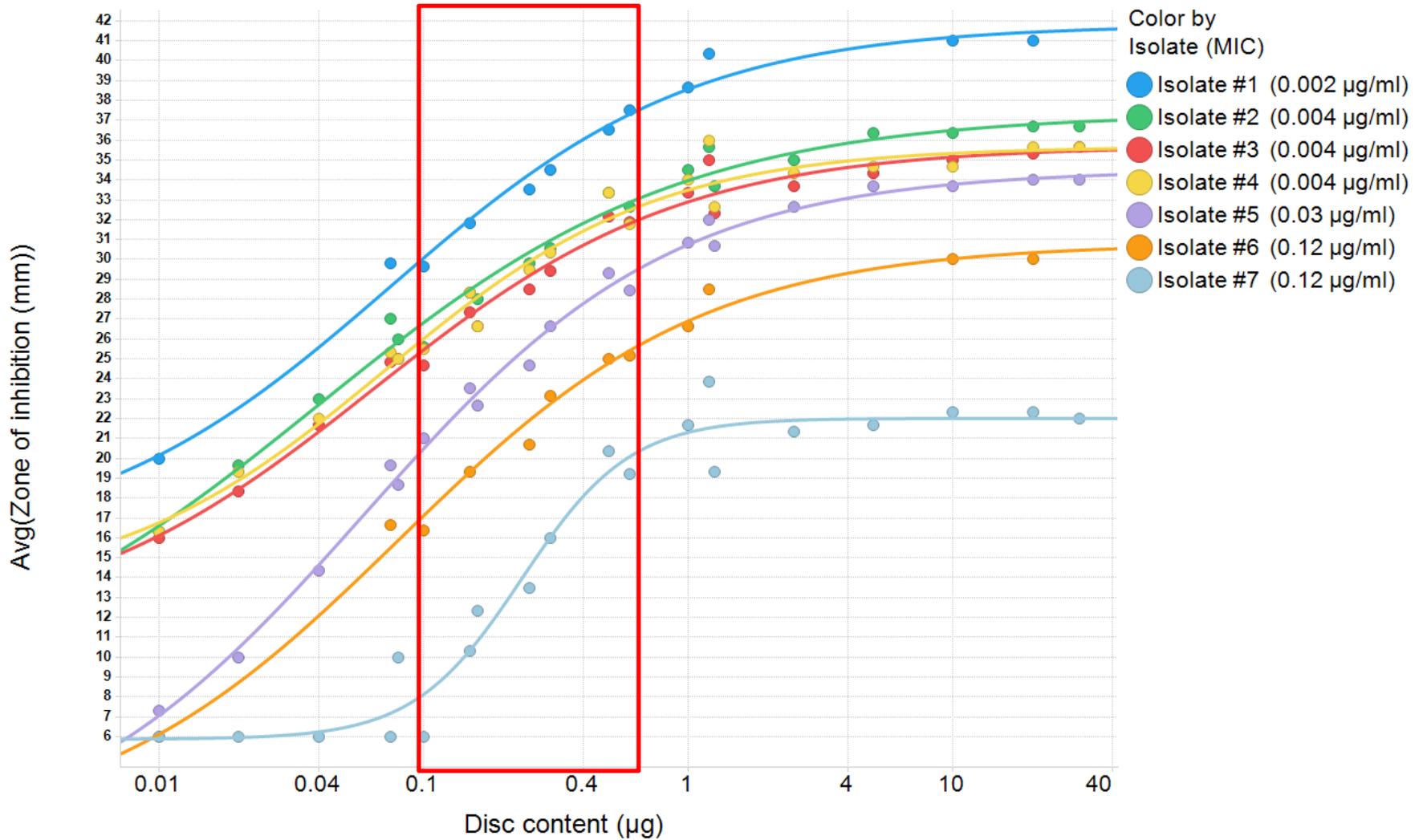


Selecting disk potency

- Optimized inhibition zone size
- Calibration of inhibition zones to reference MIC
 - Correlation between zone diameters and MIC
 - Separation between wild-type and non-wild type isolates
 - Prediction of susceptibility and resistance

Compound X Disk Mass Analysis

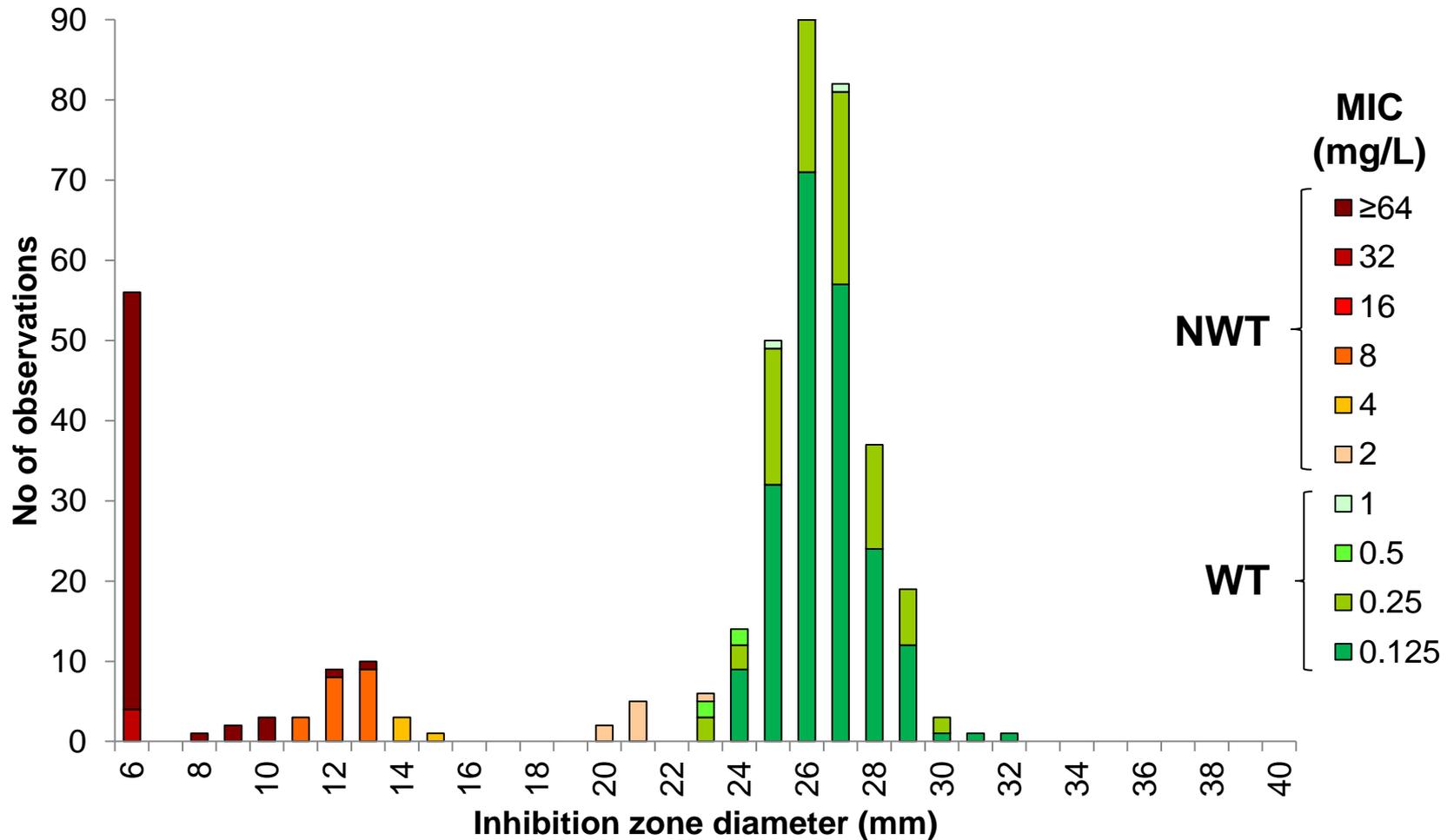
Logistic Regression Curve Fit



Inhibition zone diameter distribution with MICs as coloured bars

Agent Y, *S. aureus*

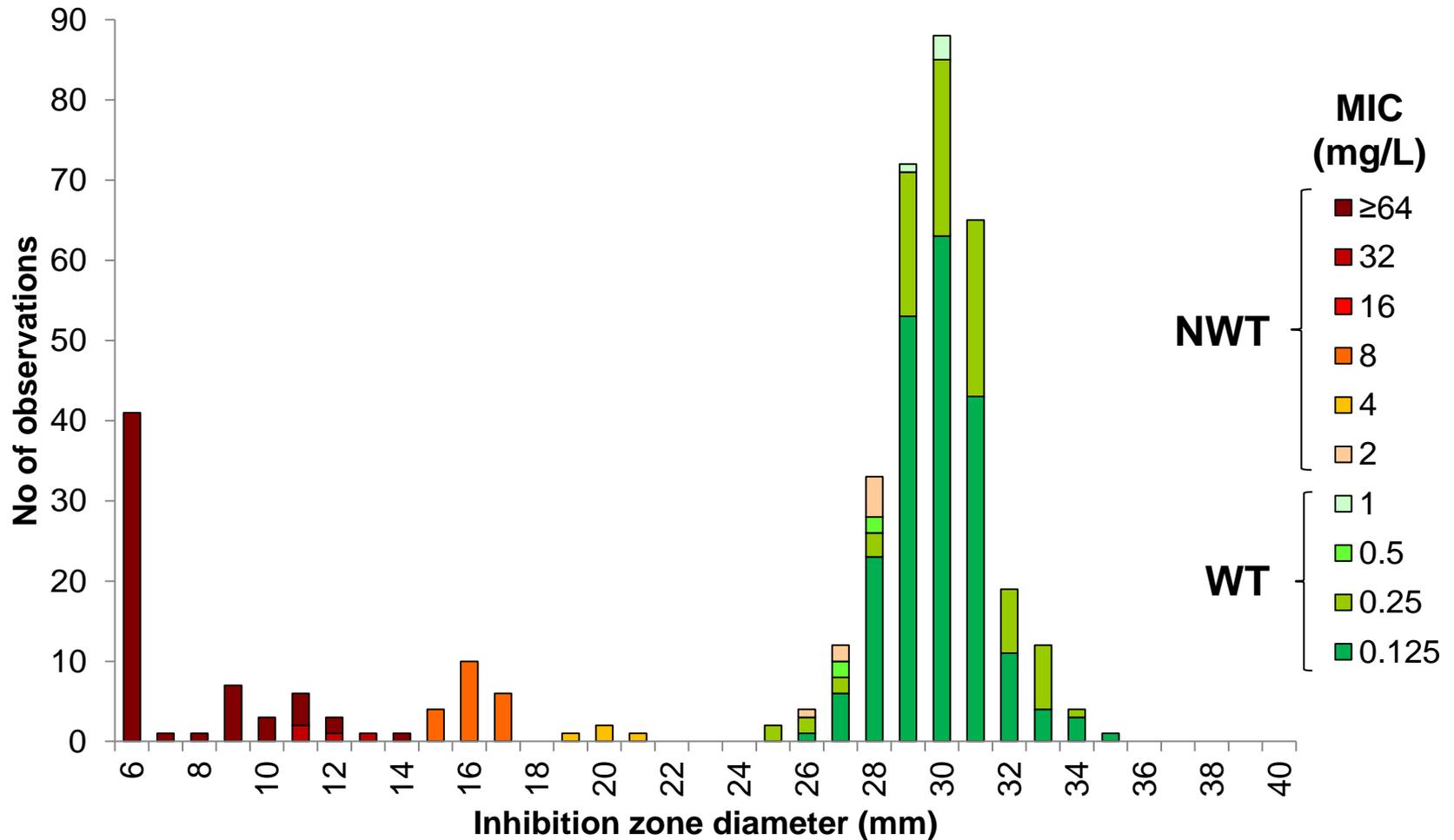
5 µg disk



Inhibition zone diameter distribution with MICs as coloured bars

Agent Y, *S. aureus*

20 µg disk



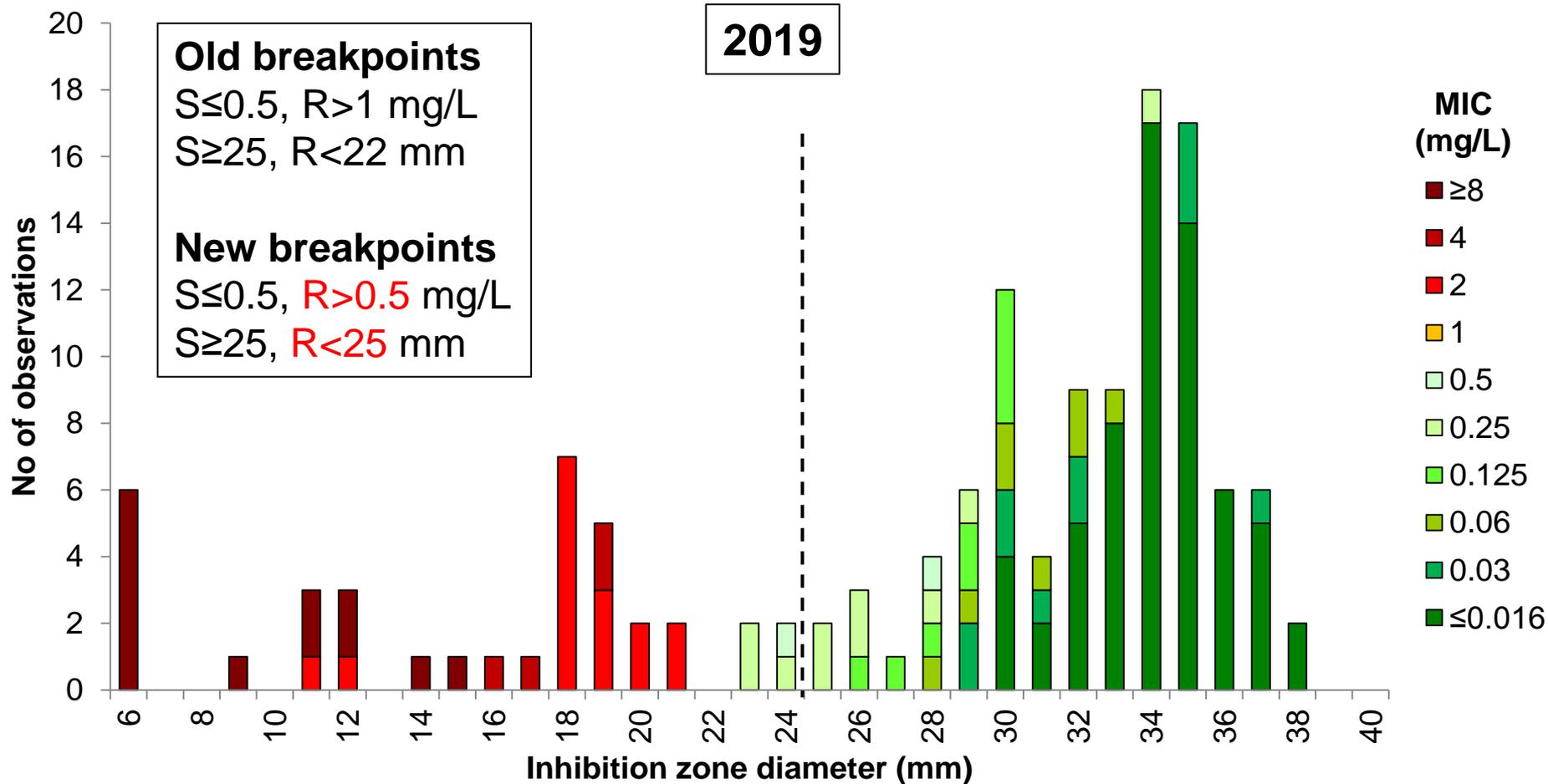
New antimicrobial agents in EUCAST BP Table 2019

- Meropenem-vaborbactam
 - Enterobacterales
 - *P. aeruginosa*
- Eravacycline
 - *E. coli*
 - *S. aureus*
 - *Enterococcus* spp.
 - Viridans group streptococci

Zone diameter break-
points and disk QC
criteria in preparation!

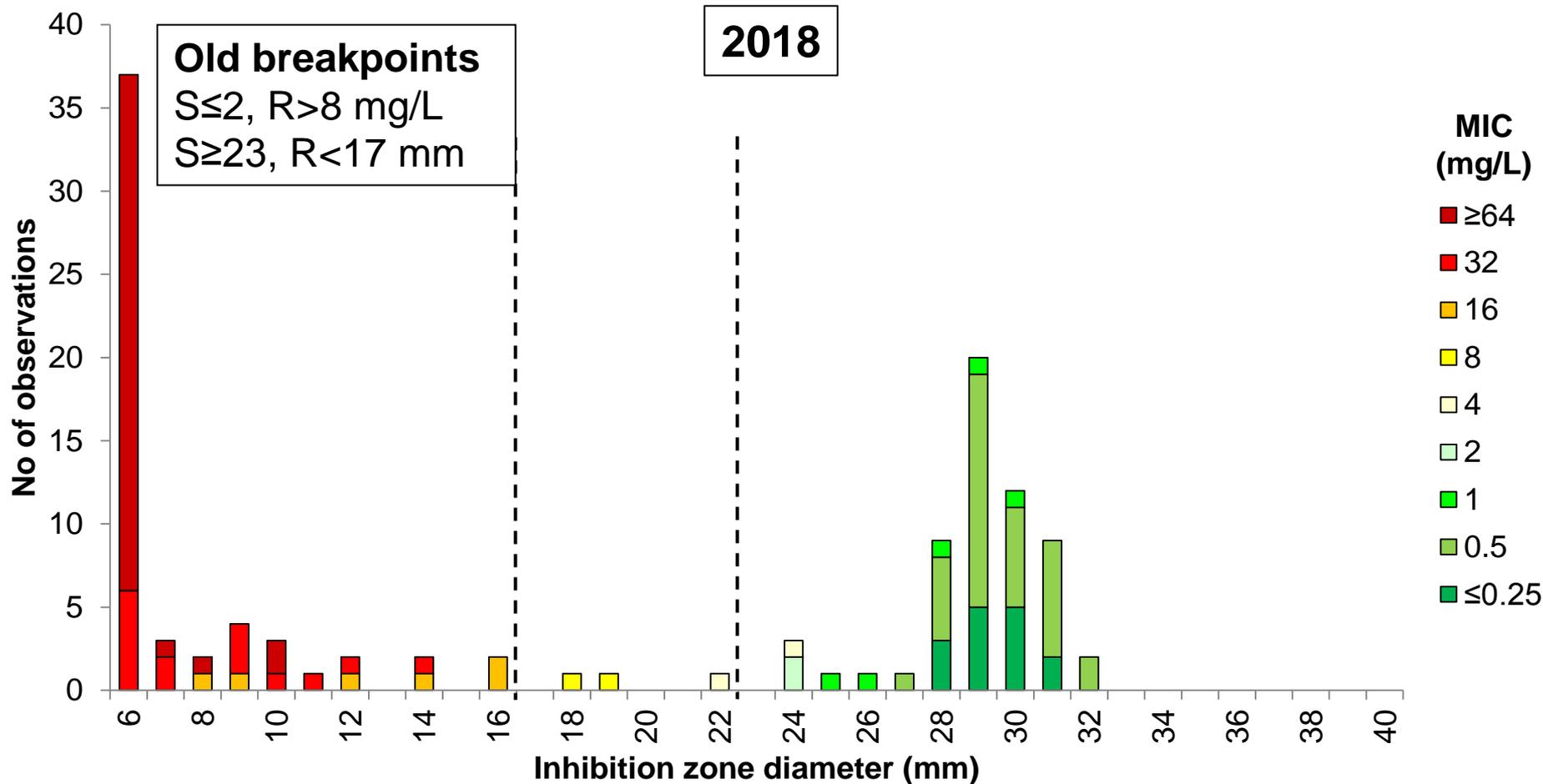
Revised carbapenem breakpoints

Ertapenem 10 µg vs. MIC Enterobacterales, 122 isolates (136 correlates)



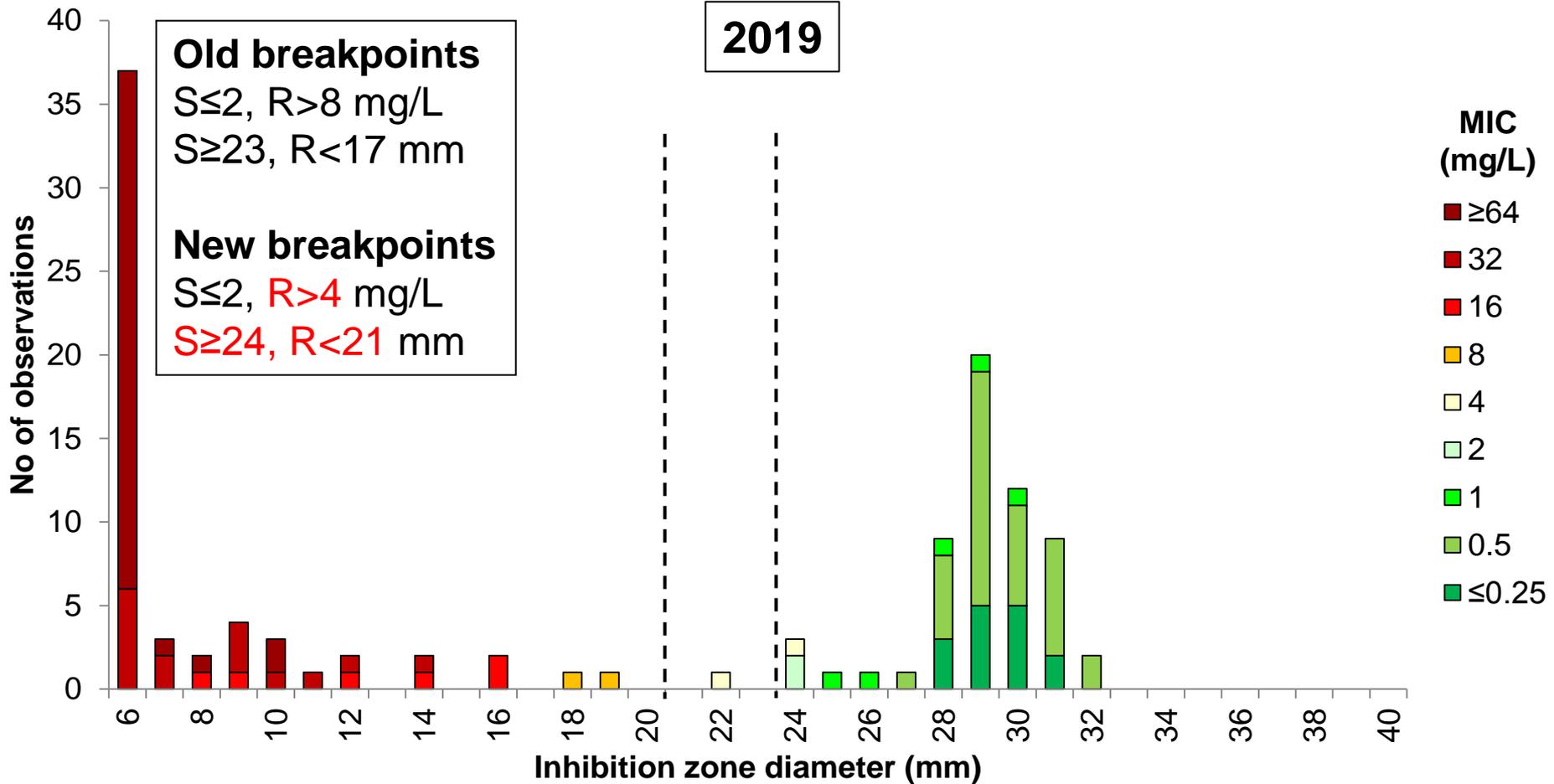
Revised carbapenem breakpoints

Imipenem 10 µg vs. MIC *Acinetobacter* spp., 119 isolates



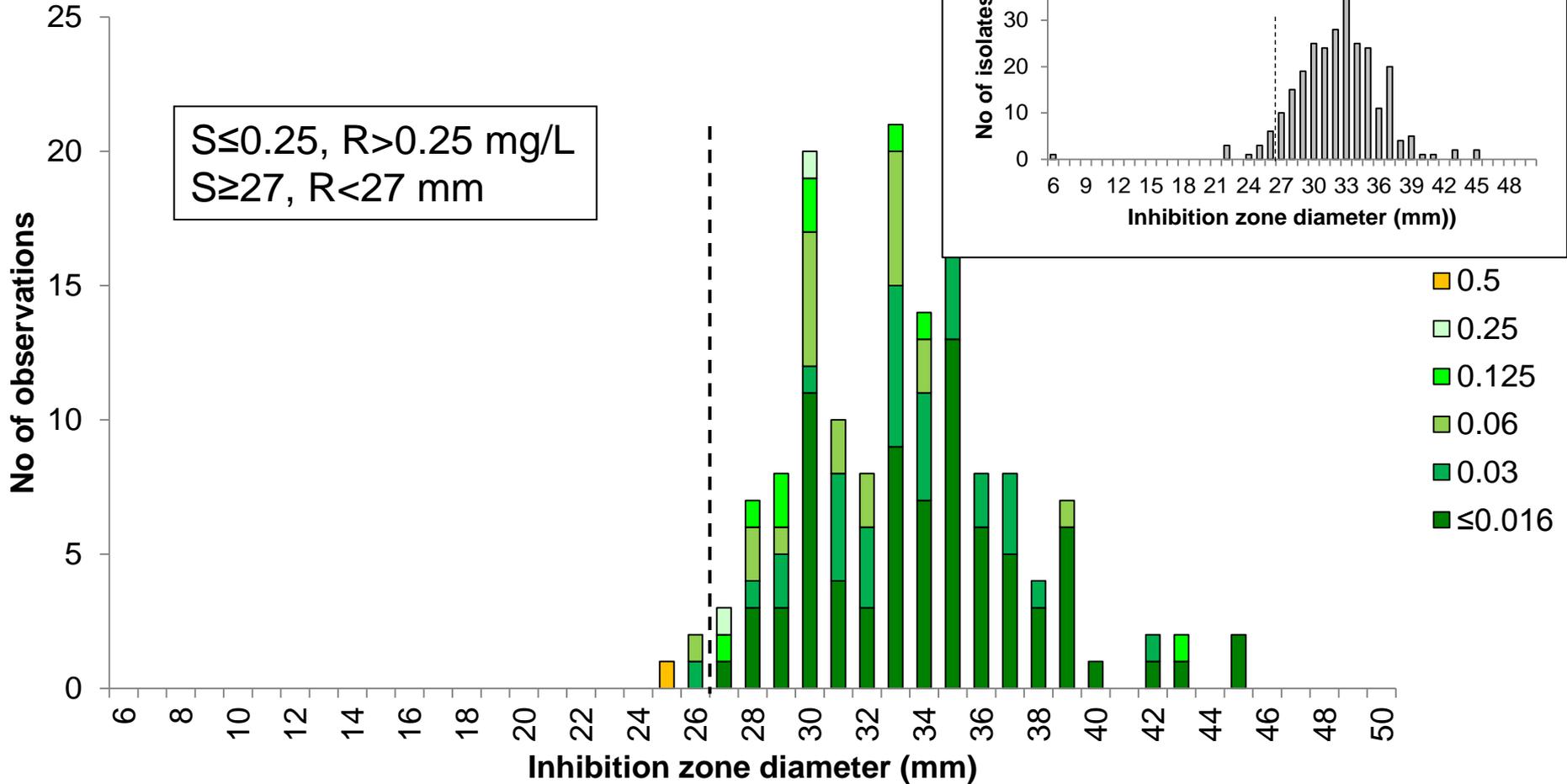
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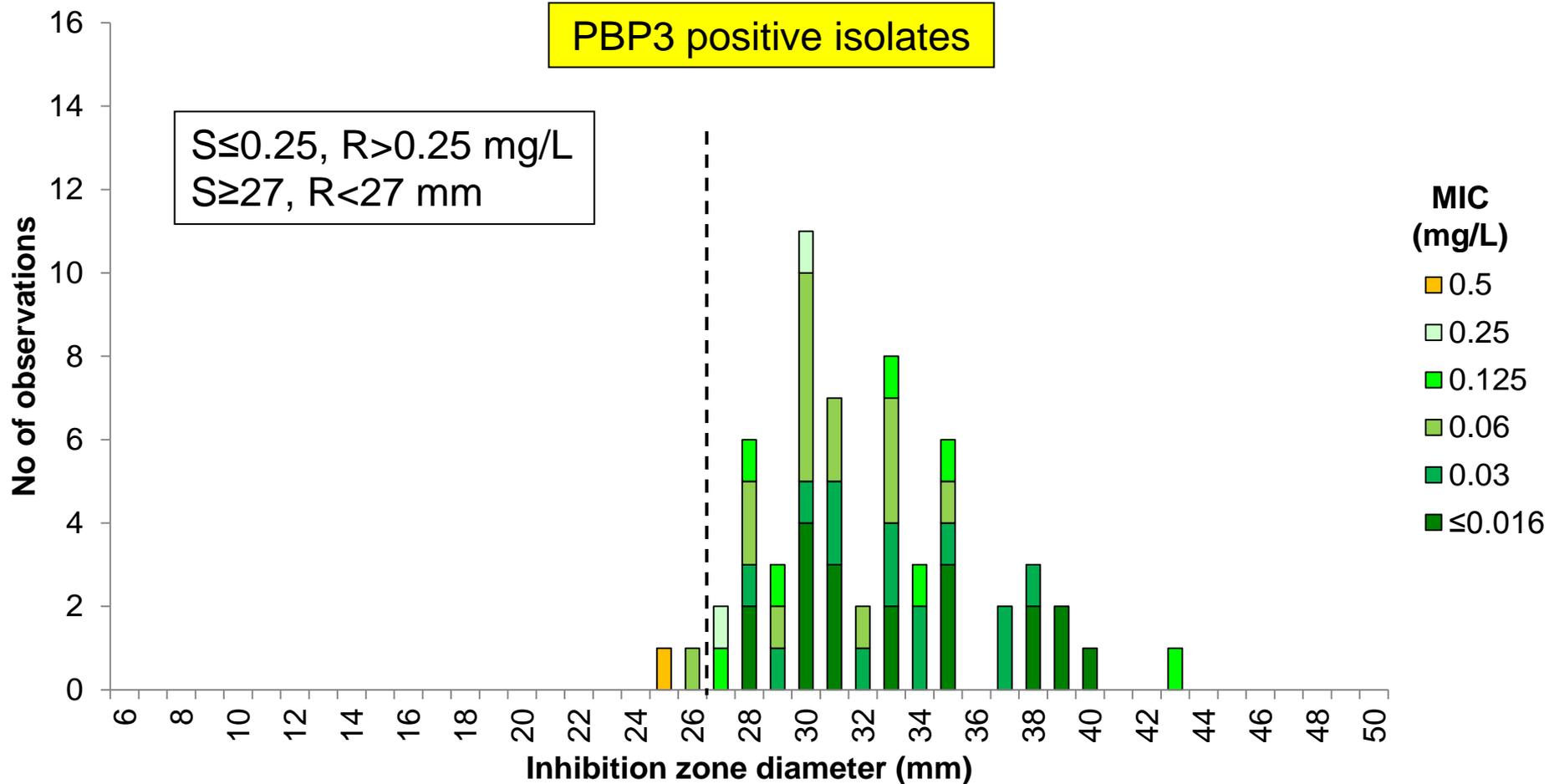
New breakpoints for *H. influenzae* and piperacillin-tazobactam

Piperacillin-tazobactam 30-6 µg
H. influenzae, 149 isolates



New breakpoints for *H. influenzae* and piperacillin-tazobactam

Piperacillin-tazobactam 30-6 µg vs. MIC *H. influenzae*, 59 isolates



New and revised warnings

AST of bacteria

Organization

EUCAST News

New definitions of S, I and R

Clinical breakpoints and dosing

Rapid AST in blood cultures

Expert rules and intrinsic resistance

Resistance mechanisms

Guidance documents

Consultations - New!

MIC and zone distributions and ECOFFs

AST of bacteria

Media preparation

MIC determination

Disk diffusion methodology

Disk diffusion implementation

Breakpoint tables

QC Tables

Calibration and validation

Warnings!



... Warnings!

EUCAST warnings concerning antimicrobial susceptibility testing products or procedures.

The EUCAST disk diffusion development laboratories, a network of laboratories coordinated from the EUCAST development laboratory in Växjö, Sweden, from time to time discover products (disks, media batches, gradient tests or procedures) which are not performing to the expected standard. When this is the case we inform the manufacturer and publish a warning on this page.

We do not systematically test all products so the lack of a warning does not imply that there is no problem with the product in question.

Laboratories which experience problems with a susceptibility test method, and suspect that this may be related to a particular product, may contact EUCAST for advice.

1. Problems with piperacillin-tazobactam gradient tests from two manufacturers.

Warning partially lifted - see below.

2. **Wide variation in disk quality in 16 disks from nine manufacturers** - this warning was issued in 2015 and reiterated 2016 and 2017. There is new information on improved quality November 23, 2017 (see below).

www.eucast.org

New and revised warnings

- Meropenem BMD
 - Several batches of DKMGN Sensititre plates (Thermo Fisher Scientific) with inoculum 1×10^5 CFU/mL underestimates meropenem MICs for some isolates of *E. coli* and *K. pneumoniae*.
 - The use of a higher inoculum results in correct MICs (see technical bulletin from manufacturer)

Meropenem MICs on DKMGN

Example

Escherichia coli (VIM-1)

Plate	Plate lot	MER
DKMGN*	1	2
DKMGN*	1	16
DKMGN*	1	4/16
DKMGN*	1	2
DKMGN*	2	8
DKMGN*	2	2/8
DKMGN*	2	4
DKMGN*	3	8
DKMGN*	3	4
DKMGN*	3	2

* 10 µL transfer to broth = 1×10^5 CFU /mL

Plate	Plate lot	MER**
Enterobacterales std*	1	>16
Enterobacterales std*	2	>16
Enterobacterales std*	2	>16
Enterobacterales std*	3	>16
Pseudomonas std*	1	32
Gram-neg extended*	1	>16

* 50 µL transfer to broth = 5×10^5 CFU /mL

** MER MIC confirmed at 32 mg/L at external lab

- QC was within range for meropenem for all lots of DKMGN plates (*P. aeruginosa* ATCC 28753).
- It was confirmed by Thermo Fisher that a higher inoculum on the DKMGN panel resulted in higher MICs.

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 - The use of a higher inoculum results in correct MICs (see technical bulletin from manufacturer)
- Vancomycin gradient tests (collaboration with K-res Tromsø)
 - Gradient tests from three manufacturers underestimate vancomycin MICs in low-level resistant *E. faecalis* and *E. faecium* with *vanB*

Enterococci and vancomycin EDL study

- *E. faecalis* and *E. faecium* (Tromsø, Norway)
 - 55 isolates, of which 25 *vanB* positive
- BMD read after 24 h as reference (ISO 20776-1)
- Methods evaluated
 - BMD (16-20 h)
 - Gradient tests (16-20 and 24 h)
 - Etest (bioMérieux)
 - MTS (Liofilchem)
 - MICE (Thermo Fisher Scientific)

Essential and categorical agreement for clinical isolates of enterococci, using BMD 24 h as reference

	BMD	Etest		MTS		MICE	
	16-20h	16-20h	24h	16-20h	24h	16-20h	24h
Essential agreement (total number = 44)							
EA	44	35	36	30	32	26	32
Categorical agreement (total number = 55)							
CA	51	39	40	36	39	40	49
ME	0	0	0	0	0	0	1
VME	4	16	15	19	16	15	5

EA = Essential Agreement (within ± 1 dilution of reference)

CA = Categorical Agreement

ME = Major Error (false resistant)

VME = Vey Major Error (false susceptible)

Summary enterococci and vancomycin

- All three gradient test brands underestimated vancomycin MICs for *E. faecalis* and *E. faecium* with *vanB* genes
 - The same results were obtained in independent studies performed by K-res
 - Prolonging incubation to 24 h improved results only for MICE
- BMD read at 16-20 h also underestimated vancomycin MICs to some extent
- Disk diffusion with estimation of the zone edge correctly detected all *vanB*-positive isolates, and worked well as a screening method for presence of *vanB* genes.
 - Vancomycin resistance was somewhat overestimated with the disk diffusion test.

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 - Gradient tests from three manufacturers underestimate vancomycin MICs in low-level resistant *E. faecalis* and *E. faecium* with *vanB*
- Piperacillin-tazobactam gradient tests
 - The newly developed Etest (bioMérieux) works satisfactorily for Enterobacterales and Pseudomonas

Piperacillin-tazobactam gradient tests

- 2015: EUCAST warning based on poor correlation with BMD and batch-to-batch variation for piperacillin-tazobactam gradient tests from bioMérieux (Etest) and Liofichem (MTS).
- 2019: Evaluation of the new piperacillin-tazobactam Etest using the same isolates that were used when the warning was issued, plus additional isolates with MICs close to the breakpoints (*E. coli*, *K. pneumoniae* and *P. aeruginosa*).
 - Parallel tests on Oxoid and BBL Mueller-Hinton agar.

Piperacillin-tazobactam Etest

Correlation with BMD for clinical isolates

E. coli (n=21) and *K. pneumoniae* (n=12) (66 correlates)

		PTZ BMD							
		2	4	8	16	32	≥64		
PTZ Etest	2	4	10					VME	0
	4		2					ME	2
	8				4			mE	19
	16			4	17	8			
	32			2	3	8	4		
	64								

P. aeruginosa (n=9) (18 correlates)

		PTZ BMD						
		4	8	16	32	64		
PTZ Etest	4		4				VME	2
	8						ME	0
	16			8	2			
	32				2			
	64				2			

Summary piperacillin-tazobactam Etest

- The new piperacillin-tazobactam Etest worked satisfactorily for *E. coli*, *K. pneumoniae* and *P. aeruginosa*
- bioMérieux has validated the product for Enterobacterales, Pseudomonas and Acinetobacter.
 - Note that the product is not validated for anaerobes or *H. influenzae*!
- Liofilchem is re-evaluating their piperacillin-tazobactam gradient test.

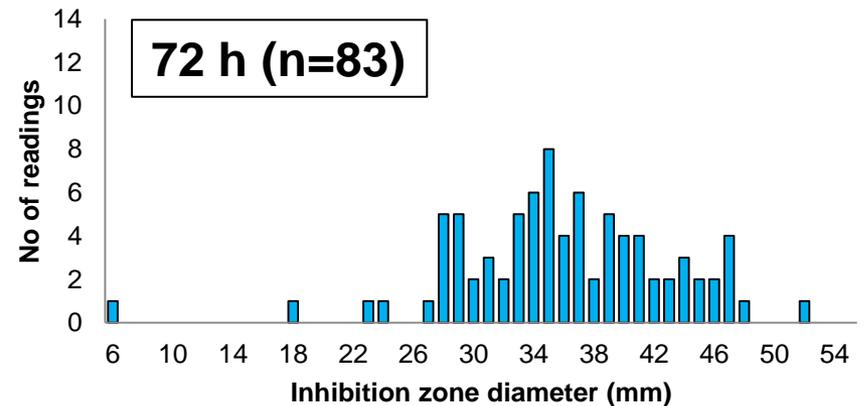
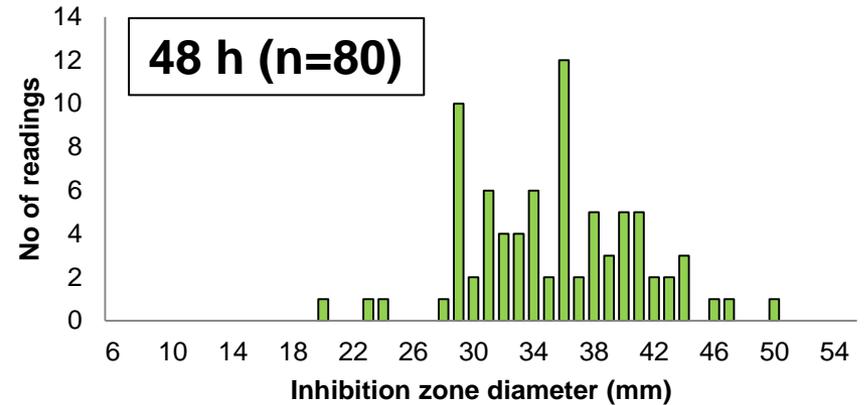
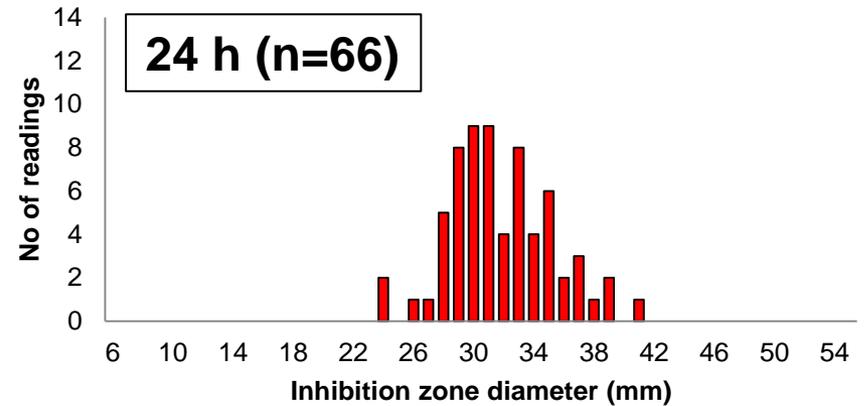
Organisms without methods and/or breakpoints

- Disk diffusion method of anaerobes (Fastidious Anaerobe Agar, FAA)
 - Validation of *Bacteroides* as part of the NordicAST study
 - Calibration of disk diffusion for additional species vs. agar dilution
 - *Prevotella* spp., *Clostridium perfringens*, *Fusobacterium necrophorum* and *Cutibacterium (Propionibacterium) acnes*
- *Nocardia* spp.
 - DD according to EUCAST method for fastidious organisms
 - Agar dilution on MH-F as MIC reference method
 - Poor growth in broth (MH and MH-F broth)

Preliminary results *Nocardia* spp.

Poster ECCMID 2019,
Rydström *et al.*

Amikacin 30 µg



Thanks!

- The EDL staff: Jenny Åhman, Amra Basic, Onur Karatuna, and Gunnar Kahlmeter
- Emma Jonasson (RAST) and Per Rydström (Nocardia), Clinical Microbiology Växjö
- Sara Petersson, Clinical Microbiology Kalmar (anaerobes)
- **All clinical laboratories participating in studies, and contributing with data and/or isolates**

Questions and/or problem?

Contact us!

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