



The new beta-lactam beta-lactamase inhibitor combinations: When and how to test?

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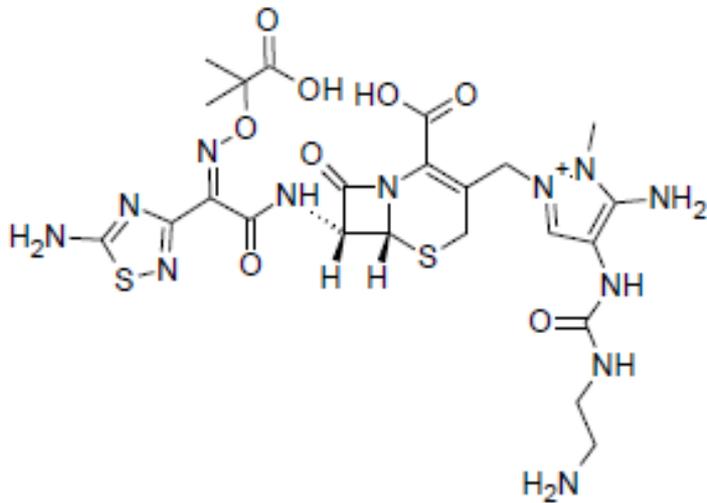
Erika Matuschek, PhD
EUCAST Development Laboratory, EDL

Overview of new and newish drugs

Antimicrobial	Name of the drug	Licensed by FDA	Licensed by EMA
Ceftolozane-tazobactam	Zerbaxa	Dec 2014	Sep 2015
Ceftazidim-avibactam	Zavicefta	Feb 2015	Apr 2016
Meropenem-vaborbactam	Vabomere	Aug 2017	Autumn 2017?
Imipenem-relebactam		Phase 3	Phase 3

Ceftolozane-tazobactam
(licensed FDA/EMA)

Ceftolozane-tazobactam



Ceftolozane

- New cephalosporin with improved PBP-affinity compared to ceftazidime
- Improved activity vs MDR *P. aeruginosa*
- No activity vs carbapenemase-producing strains
- Good activity against ESBL-producing strains, but no useful activity vs plasmid-mediated AmpC
- In *P. aeruginosa* activity vs strains with hyperproduction of AmpC

Ceftolozane-tazobactam

Antimicrobial	Indication
Ceftolozane-tazobactam	cUTI (incl. acute pyelonephritis), cIAI combined with metronidazole

In vitro activity of ceftolozane/tazobactam against clinical isolates of *Pseudomonas aeruginosa* and Enterobacteriaceae recovered in Spanish medical centres: Results of the CENIT study

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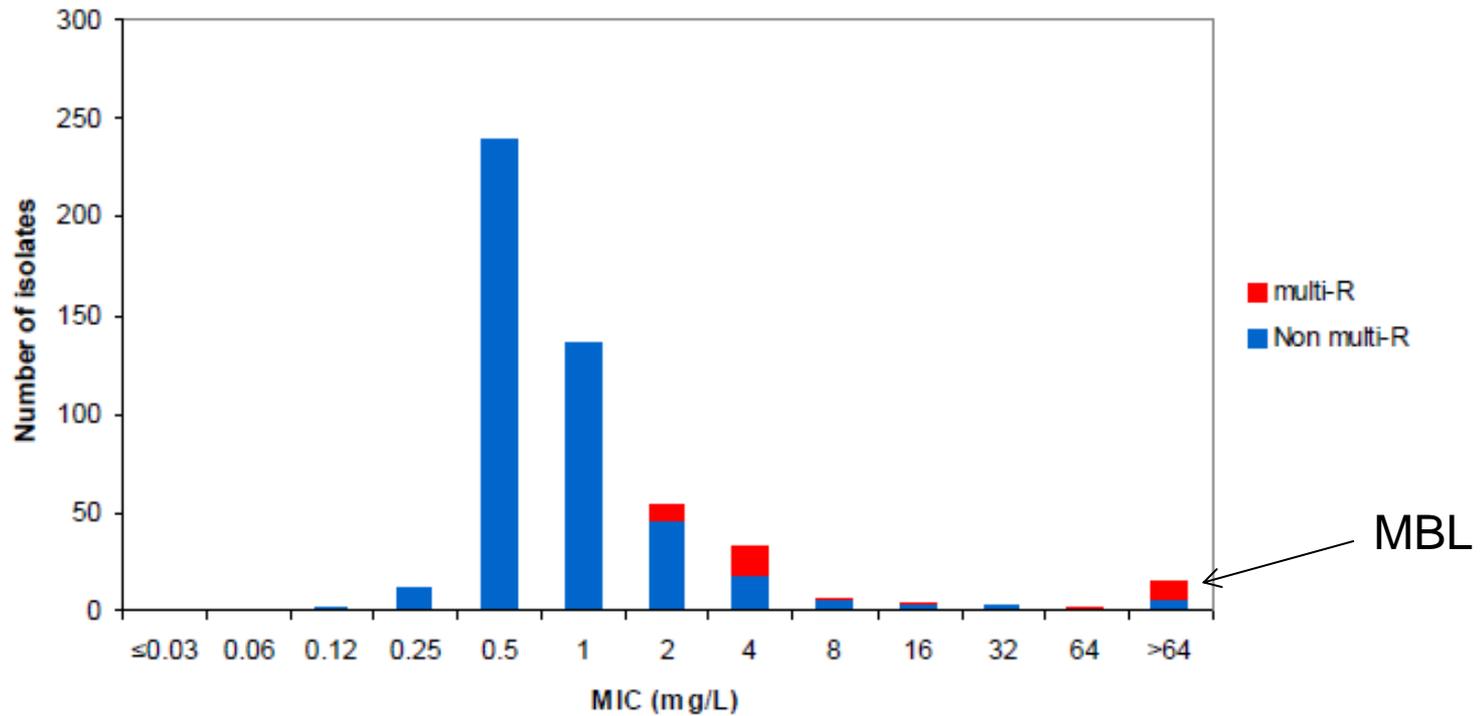
Int J Antimicrob Agents. 2015. pii: S0924-8579(15)00272-1

cUTI: complicated urinary tract infections

cIAI complicated intraabdominal infections

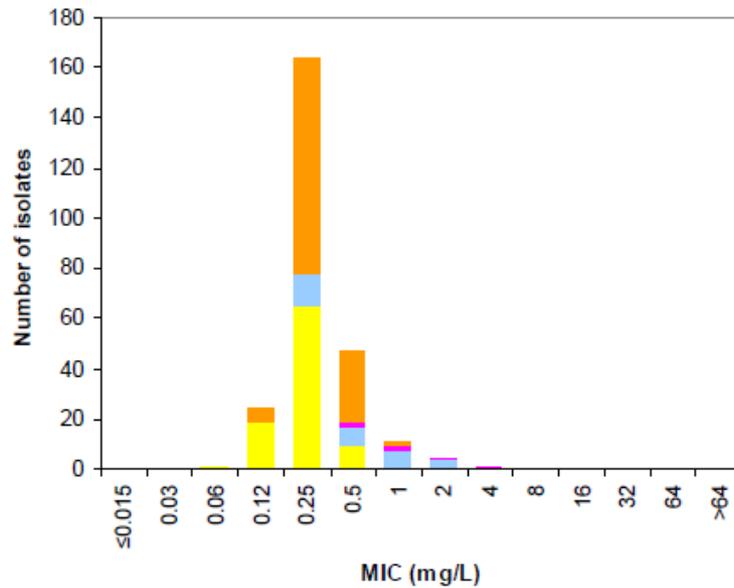
Ceftolozane-tazobactam

Pseudomonas aeruginosa

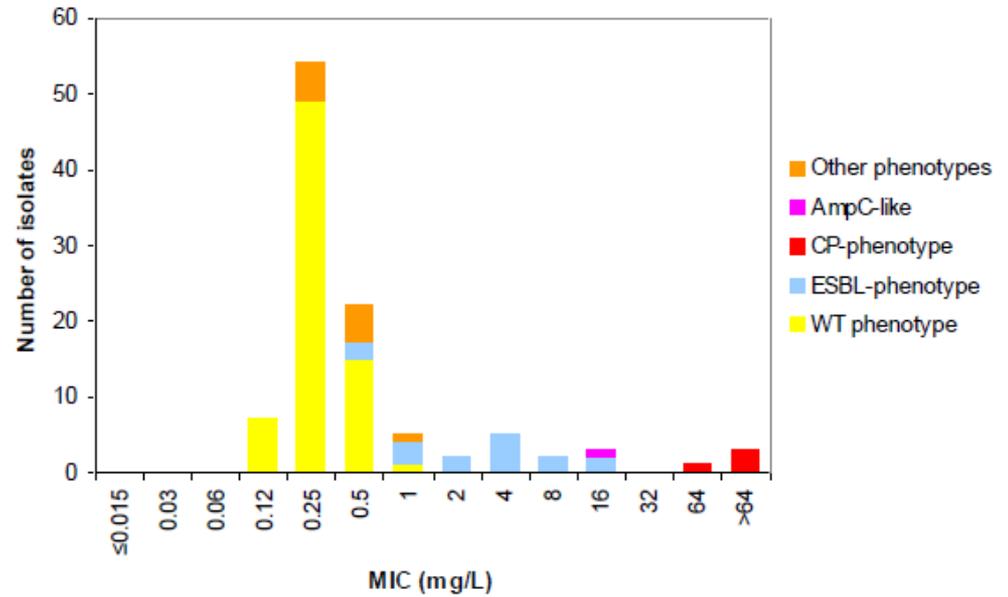


Ceftolozane-tazobactam

Escherichia coli



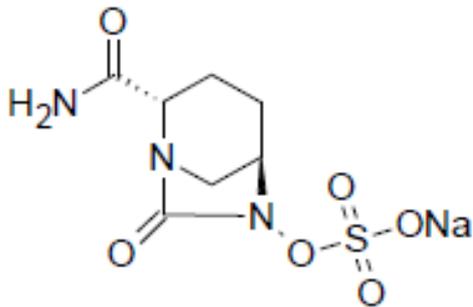
Klebsiella pneumoniae



Ceftazidime-avibactam (licensed
FDA/EMA)

Ceftazidime-avibactam

Antimicrobial	Indication
Ceftazidime-avibactam	cUTI (incl. acute pyelonephritis), cIAI combined with metronidazole



Avibactam

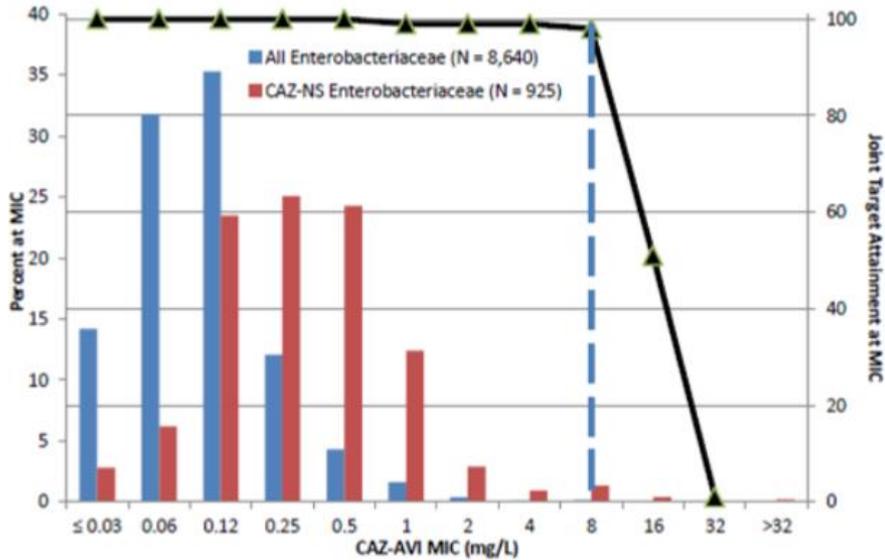
- Avibactam inhibits class A and C enzymes
- No inhibition of class B enzymes
- In vitro and clinical studies with ceftazidime, aztreonam and ceftaroline
- Only combination with ceftazidime is licensed

cUTI: complicated urinary tract infections

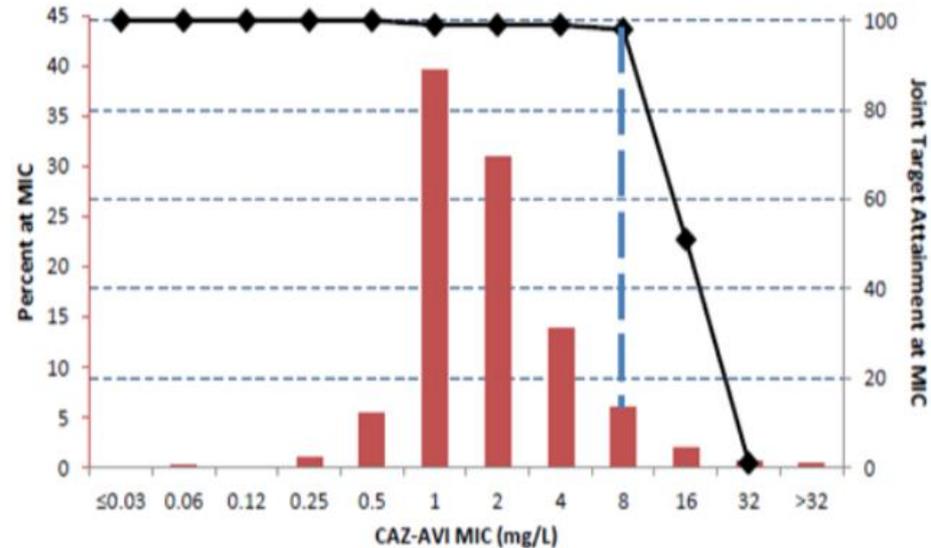
cIAI complicated intraabdominal infections

PK-PD: high target attainment for MIC 8 mg/L

Enterobacteriaceae



Pseudomonas aeruginosa



PK-PD-target: ceftazidime $fT > MIC$ 50% and inhibitor $fT > 1$ mg/L 50%
Why higher than ceftazidime? 2 g x 3 AND prolonged infusion

Clinical trials

- Resistant pathogen study
 - Multinational, multicenter, open label
 - CAZ-AVI vs best available therapy (BAT) vs ceftazidime-resistant microorganisms
 - cIAI or cUTI, dosing 2 g ceftazidime x 3 as 2 h infusion
 - Recommended BAT-alternatives: carbapenems, tigecycline, colistin (not restricted to these)
 - Efficacy end of therapy, TOC (7-10 d after last dose) and follow-up (28-35 d from randomisation)
 - Clinical cure: Complete resolution or significant improvement of signs and symptoms of the index infection
- Furthermore: 1 RCT of cIAI: ceftazidime-avibactam+metronidazole vs meropenem
- Pooled data: 79.3% with CAZ-R Enterobacteriaceae responded to CAZ-AVI, compared to 82.4% response if CAS-S
- **Problem: few carbapenemase-producers in the trials**

Emerging resistance to ceftazidime-avibactam

- *K. pneumoniae* ST258, bloodstream infection – isolates on day 1 (MIC 4 mg/L) and 2 (MIC 32 mg/L) (meropenem therapy)
- PacBio sequencing of both strains
- OmpK35 and K36 mutations
- Increase expression of KPC-3 (multiple copies of Tn4401 transposon containing KPC-3)

Resistance to Ceftazidime-Avibactam in *Klebsiella pneumoniae* Due to Porin Mutations and the Increased Expression of KPC-3

Romney M. Humphries,  Peera Hemarajata

Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, California, USA

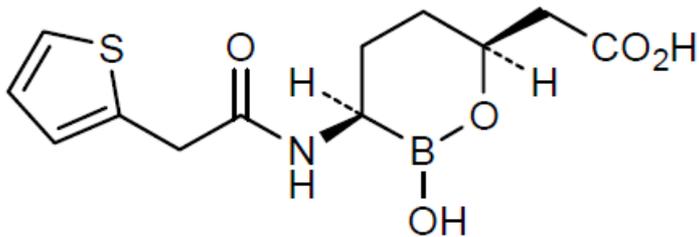
KEYWORDS ceftazidime-avibactam, KPC, OmpK35, OmpK36, resistance

AAC 2017; 61: e00537-17.

Meropenem-vaborbactam
(licensed FDA)

Meropenem-vaborbactam

Antimicrobial	Indication
Meropenem-vaborbactam	cUTI (incl. acute pyelonephritis)



Vaborbactam

- Vabomere (Rempex) licensed by FDA on 29 Aug 2017, not yet in Europe
- Non- β -lactam, non-suicide inhibitor
- Protects against class A and C beta-lactamases, not B or D
- Vaborbactam tested in fixed concentration of 8 mg/L
- Dosing: meropenem 2 g+vaborbactam 2 g x 3, 3 h infusion

cUTI: complicated urinary tract infections

Meropenem-vaborbactam clinical trial

- Clinical trial with 545 adults with cUTI, including those with pyelonephritis
 - End of intravenous treatment: 98% had cure/improvement and negative urine culture with meropenem-vaborbactam vs 94% for piperacillin-tazobactam
 - Qualified infectious disease product (QIDP) – given priority review by FDA

MICs meropenem-vaborbactam

Organism (no. of strains) and antimicrobial agent(s)	MIC ($\mu\text{g/ml}$)	
	50%	90%
KPC-producing <i>K. pneumoniae</i> (208)		
Meropenem	16	>64
Meropenem-vaborbactam ^a	≤ 0.06	1
<i>E. cloacae</i> (39)		
Meropenem	8	32
Meropenem-vaborbactam ^a	≤ 0.06	0.25
KPC-producing <i>E. coli</i> (21)		
Meropenem	4	8
Meropenem-vaborbactam ^a	≤ 0.06	≤ 0.06
KPC-producing <i>K. oxytoca</i> (14)		
Meropenem	4	32
Meropenem-vaborbactam ^a	≤ 0.06	0.5
KPC-producing <i>C. freundii</i> (12)		
Meropenem	4	8
Meropenem-vaborbactam ^a	≤ 0.06	0.12
Isolates carrying multiple β -lactamases (75)		
Meropenem	32	>64
Meropenem-vaborbactam ^a	≤ 0.06	1

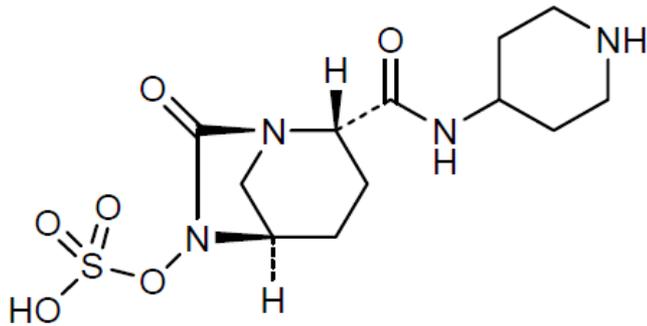
Castanheira M, et al. 2016.
Antimicrob Agents Chemother
60:5454 –5458

Breakpoint (FDA):
S ≤ 4 mg/L, R>8 mg/L

Only one dosing regimen, not clear why there is an intermediate group

Imipenem-relebactam (Phase 3)

Imipenem-relebactam



Relebactam

- Relebactam: bicyclic diazabicyclooctane, non- β -lactam, β -lactamase inhibitor of class A and class C β -lactamases
- Currently tested under a phase III clinical trial against imipenem-colistin combination in the treatment of imipenem-resistant infections (HABP, VABP, cIAIs, and cUTIs)
- Currently also tested under a phase III clinical trial against piperacillin/tazobactam in subjects with HABP or VABP
- Dosing regimen 500 mg imipenem/250 mg relebactam q6h
- 5 to 21 days for cIAI and cUTI or for 7 to 21 days for HABP or VABP

cUTI: complicated urinary tract infections
cIAI complicated intraabdominal infections

HABP: hospital acquired bacterial pneumonia
VABP: ventilator associated bacterial pneumonia

In vitro activity of imipenem-relebactam

Organism	n	Drug	MIC (mg/L)							
			≤0.5	1	2	4	8	16	32	>32
<i>P. aeruginosa</i>	1065	IMI	20.8	58.3	64.1	68.9	81.4	94.2	97.4	100
		IMI/REL	69.9	80.9	91.5	93.7	96.2	96.9	97.8	100
<i>P. aeruginosa</i> , IMI-NS	331	IMI					40.2	81.3	91.5	100
		IMI/REL	9.7	40.2	72.5	79.8	87.6	90.0	93.1	100
NPE	1949	IMI	76.3	89.3	94.0	95.6	96.5	97.3	97.7	100
		IMI/REL	87.6	95.7	97.5	98.4	98.7	98.9	99.0	100
NPE, IMI-NS	116	IMI				26.7	40.5	55.2	61.2	100
		IMI/REL	39.7	46.6	58.6	73.3	78.4	81.9	83.6	100

Shaded area indicates susceptible by EUCAST 2015 imipenem breakpoint; MIC₉₀ bolded; NPE, non-*Proteaeae Enterobacteriaceae*; IMI, imipenem; REL, relebactam; NS, non-susceptible

Lob S et al. Diagn Microbiol Infect Dis. 2017;88(2):171-176

Relebactam: fixed concentration 4 mg/L

Good activity vs KPC, mixed vs OXA-48, no activity vs MBLs

Summary of activity – all drugs

Antimicrobial	EBA ESBL	EBA AmpC	KPC KP	EBA MBL	PA MDR	AB MDR	SM
Ceftolozane-tazobactam	+	-	-	-	+	-	-
Ceftazidime-avibactam	+	+	+	-	+/-	-	-
Meropenem-vaborbactam	+	+	+	-	+/-	-	-
Imipenem-relebactam	+	+	+	-	+/-	-	-

Adapted from Falagas ME et al. Expert Review of Anti-infect. Ther. 2016; 8:747-763

Quality control of BL/BLI combinations

Recommendations for MIC determination and disk diffusion (EUCAST QC Tables v 8.0):

- The active component is checked with a susceptible QC strain.
- Specific β -lactamase-producing strains are recommended to check the inhibitor component of β -lactam- β -lactamase inhibitor combinations.
- Both tests should be part of the routine QC.

http://www.eucast.org/ast_of_bacteria/qc_tables/

QC recommendations for active component

Example Enterobacterales

Escherichia coli ATCC 25922

(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target	Range		Target	Range
Amoxicillin-clavulanic acid	4	2-8	20-10	21	18-24
Ceftazidime-avibactam	0.125-0.25	0.06-0.5	10-4	27	24-30
Ceftolozane-tazobactam	0.25	0.125-0.5	30-10	28	24-32
Piperacillin-tazobactam	2	1-4	30-6	24	21-27

Fixed concentration of the inhibitor

- Clavulanic acid 2 mg/L
- Avibactam 4 mg/L
- Tazobactam 4 mg/L

Strains to control of the inhibitor

- Clavulanic acid: *E. coli* ATCC 35218 (TEM-1 β -lactamase)
- Avibactam: *K. pneumoniae* ATCC 700603 (SHV-18 ESBL producer)
- Tazobactam: *E. coli* ATCC 35218 or *K. pneumoniae* ATCC 700603

QC recommendations for inhibitor

Escherichia coli ATCC 35218

(NCTC 11954, CIP 102181, DSM 5923, CCUG 30600, CECT 943)

TEM-1 β -lactamase-producing strain (non-ESBL)

Antimicrobial agent	MIC (mg/L)		Disk content (μ g)	Inhibition zone diameter (mm)	
	Target	Range		Target	Range
Amoxicillin-clavulanic acid	8-16	4-32	20-10	19-20	17-22
Ceftolozane-tazobactam	0.125	0.06-0.25	30-10	28	25-31
Piperacillin-tazobactam	1	0.5-2	30-6	24	21-27

Klebsiella pneumoniae ATCC 700603

(NCTC 13368, CCUG 45421, CECT 7787)

SHV-18 ESBL producer

Antimicrobial agent	MIC (mg/L)		Disk content (μ g)	Inhibition zone diameter (mm)	
	Target	Range		Target	Range
Ceftazidime-avibactam	0.5-1	0.25-2	10-4	21	18-24
Ceftolozane-tazobactam	1	0.5-2	30-10	21	17-25
Piperacillin-tazobactam	16	8-32	30-6	17	14-20

..and don't forget amox-clav 2-1 µg!

***Haemophilus influenzae* ATCC 49766**

(NCTC 12975, CIP 103570, DSM 11970, CCUG 29539)

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target ¹	Range ²		Target ¹	Range ²
Amoxicillin-clavulanic acid	0.25	0.125-0.5	2-1	20	17-23

Fixed concentration of the inhibitor

- Clavulanic acid 2 mg/L

Strains to control of the inhibitor

- *E. coli* ATCC 35218 (MIC) and *S. aureus* ATCC 29213 (disk diffusion)

***Staphylococcus aureus* ATCC 29213**

(NCTC 12973, CIP 103429, DSM 2569, CCUG 15915, CECT 794)

β-lactamase-producing strain (weak)

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target	Range		Target	Range
Amoxicillin-clavulanic acid	Note ⁹	Note ⁹	2-1	22	19-25

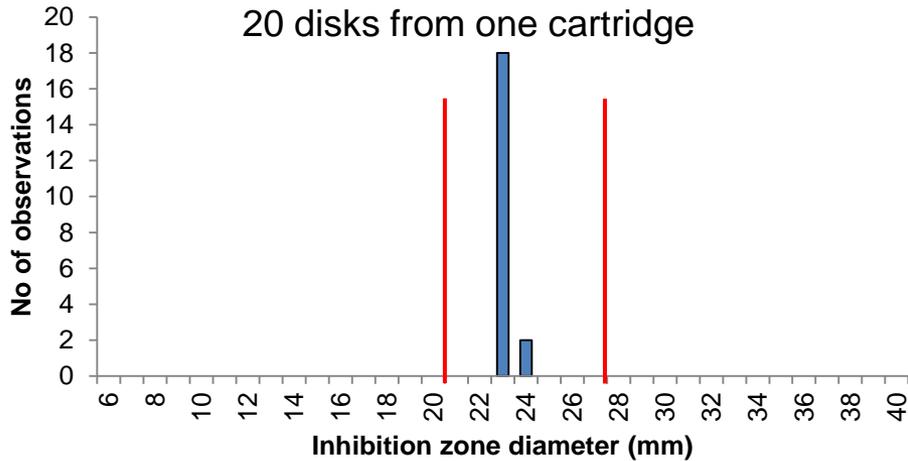
Piperacillin-tazobactam 30-6 µg

Control of active component

Control of inhibitor

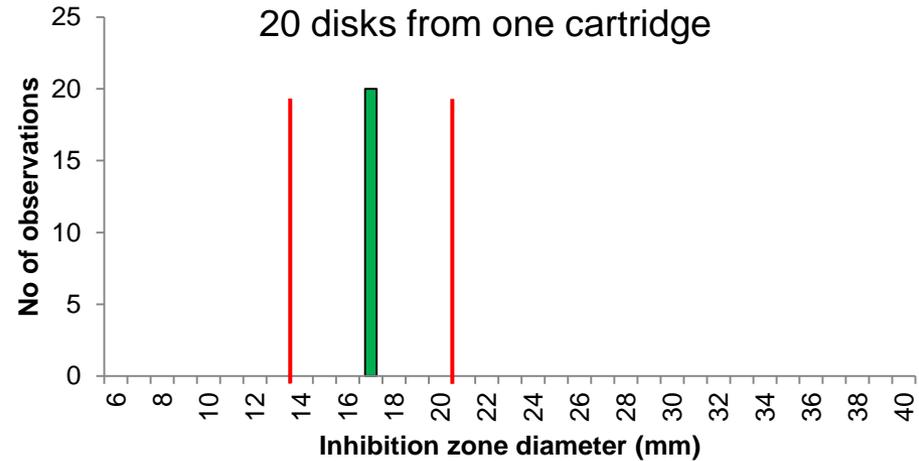
E. coli ATCC 25922, Oxoid disks

20 disks from one cartridge



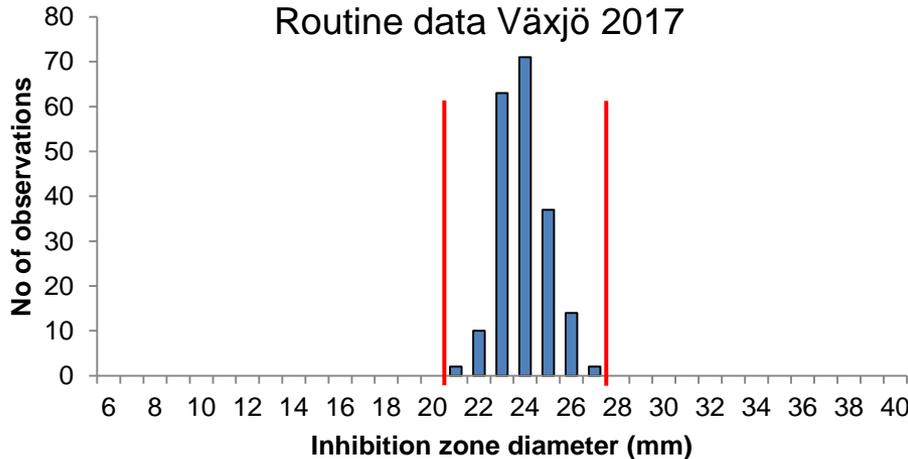
K. pneumoniae ATCC 700603, Oxoid disks

20 disks from one cartridge



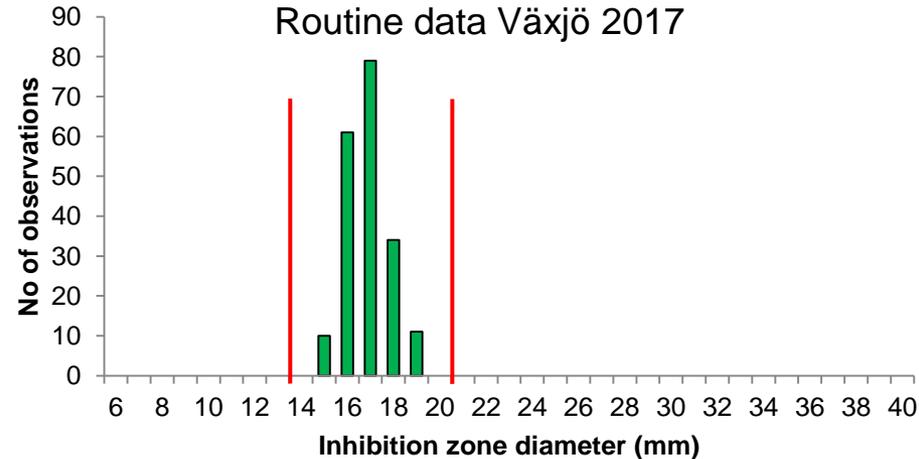
E. coli ATCC 25922, Oxoid disks

Routine data Växjö 2017



K. pneumoniae ATCC 700603, Oxoid disks

Routine data Växjö 2017



Example QC data BMD panels

EDL Enterobacteriaceae panel

Control of piperacillin

Control of tazobactam

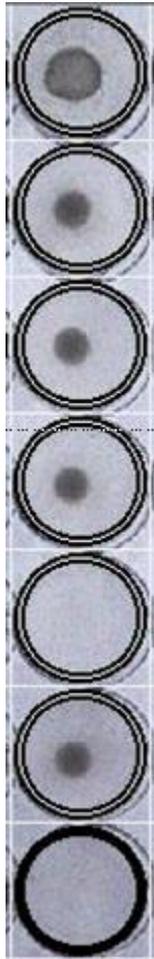
<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 278583	<i>K. pneumoniae</i> ATCC 700603
4	4	
4	8	
2	4	
2	4	16
4	4	16
2	4	16
2	4	16
2	4	16
4	4	16
4	8	16
4	8	16
4	8	16
4	8	16
4	4	16
2	4	16
2	4	16
2	4	16

Target Range

2	2-4	16
1-4	1-8	8-32

On target
 Upper limit
 Lower limit
 Out of range

Skipped wells



0.5

1

2

4

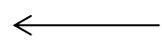
8

16

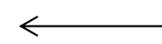
32

Clinical breakpoints

Enterobacterales and piperazillin-tazobactam
 $S \leq 8$, $R > 16$ mg/L



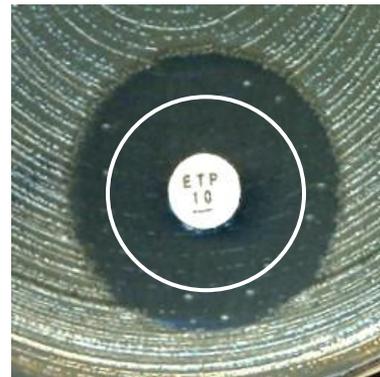
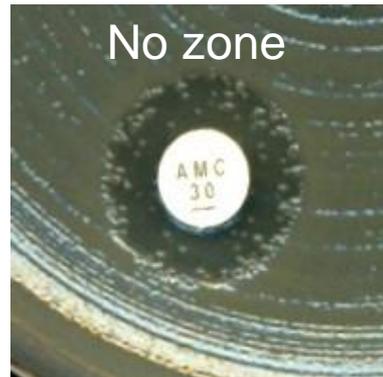
8 mg/L, susceptible?



32 mg/L, resistant?

Colonies within zone

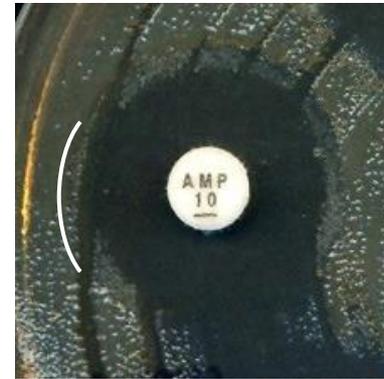
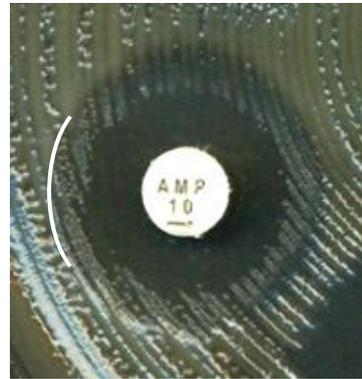
- In case of distinct colonies within zones, check for purity and repeat the test if necessary.
- If cultures are pure, colonies within zones should be taken into account when measuring the diameter.



Reading of zones with colonies within the zone.

Enterobacteriaceae with ampicillin, ampicillin-sulbactam and amoxicillin-clavulanic acid

- Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars. The inner zone is not seen with some batches of agar and when the outer zone is read there is no difference between batches.



Gradient tests for testing BL/BLI combinations

- Amoxicillin-clavulanic acid
 - Strips with fixed concentration of clavulanic acid can only be bought from Liofilchem
- Piperacillin-tazobactam
 - Warning on the EUCAST website
- Ceftolozane-tazobactam and ceftazidime-avibactam gradient tests are available
 - No EUCAST evaluation performed
 - The fact that there is no warning on the EUCAST website does not mean that there might not be problems with these products

Organization

EUCAST News

Clinical breakpoints

Expert rules and intrinsic resistance

Resistance mechanisms

Guidance documents

MIC distributions and ECOFFs

Zone distributions and ECOFFs

AST of bacteria

Media preparation

MIC determination

Disk diffusion methodology

Disk diffusion implementation

Compliance of manufacturers

Breakpoint tables

QC Tables

Calibration and validation

Warnings!

Guidance documents

Projects and data submission

MIC testing services from EUCAST

Previous versions of documents

AST of mycobacteria

AST of fungi

AST of veterinary pathogens

Frequently Asked Questions (FAQ)

Meetings

Presentations and statistics

Documents

Translations



... 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 piperacillintazobactam gradient tests from two manufacturers (see below).
2. Wide variation in disk quality in 16 disks from nine manufacturers (see below)

1. Problems with piperacillintazobactam gradient tests from two manufacturers

Following reports of problems related to piperacillin-tazobactam gradient tests, several batches of tests from bioMérieux and Liofilchem (from both single packs and multipacks) were evaluated at the EUCAST Development Laboratory, Växjö, Sweden (January 2016). Enterobacteriaceae and *Pseudomonas aeruginosa* (type strains and strains with resistance mechanisms) and broth micro dilution were used.

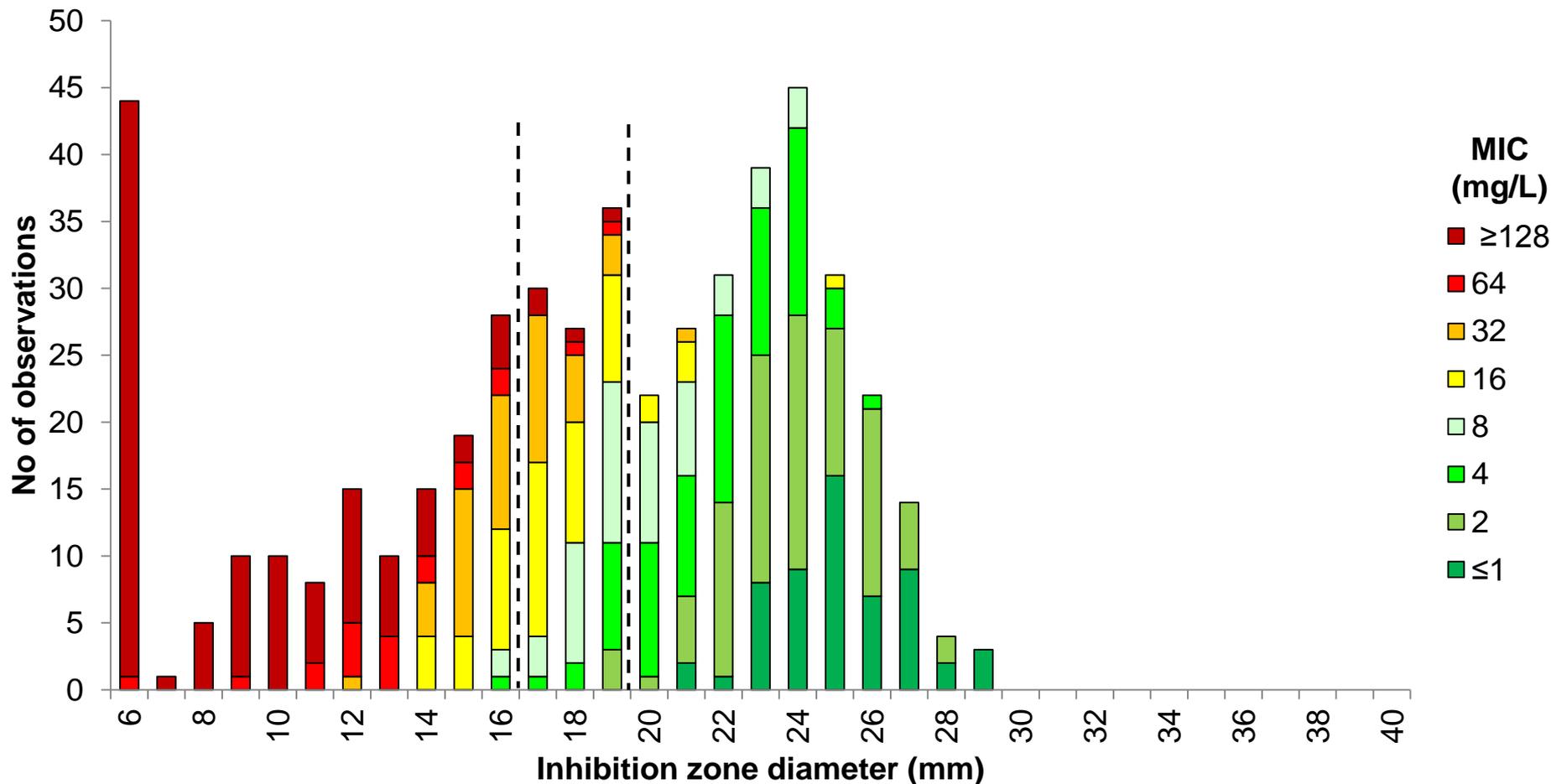
Gradient tests from both manufacturers gave variable and unreliable results. The difference between gradient tests and broth micro dilution was not systematic, some values were two dilutions too high, others too low. There was also considerable variation between batches from the same manufacturer.

We urge manufacturers to seriously consider these problems and users to introduce internal quality control of each procedure. We have detected no reason not to trust the disk diffusion (30+6 µg disk) results but currently you can not confirm your disk diffusion results with a gradient test.

EUCAST recommendations

- Use disk diffusion or broth microdilution for AST of BL/BLI combinations
- Disk diffusion
 - Zone diameter breakpoints are set to avoid reporting isolates as false susceptible
- BMD
 - Commercial products available
 - EDL performs BMD on requests (see <http://www.nordicast.org/metoder>)

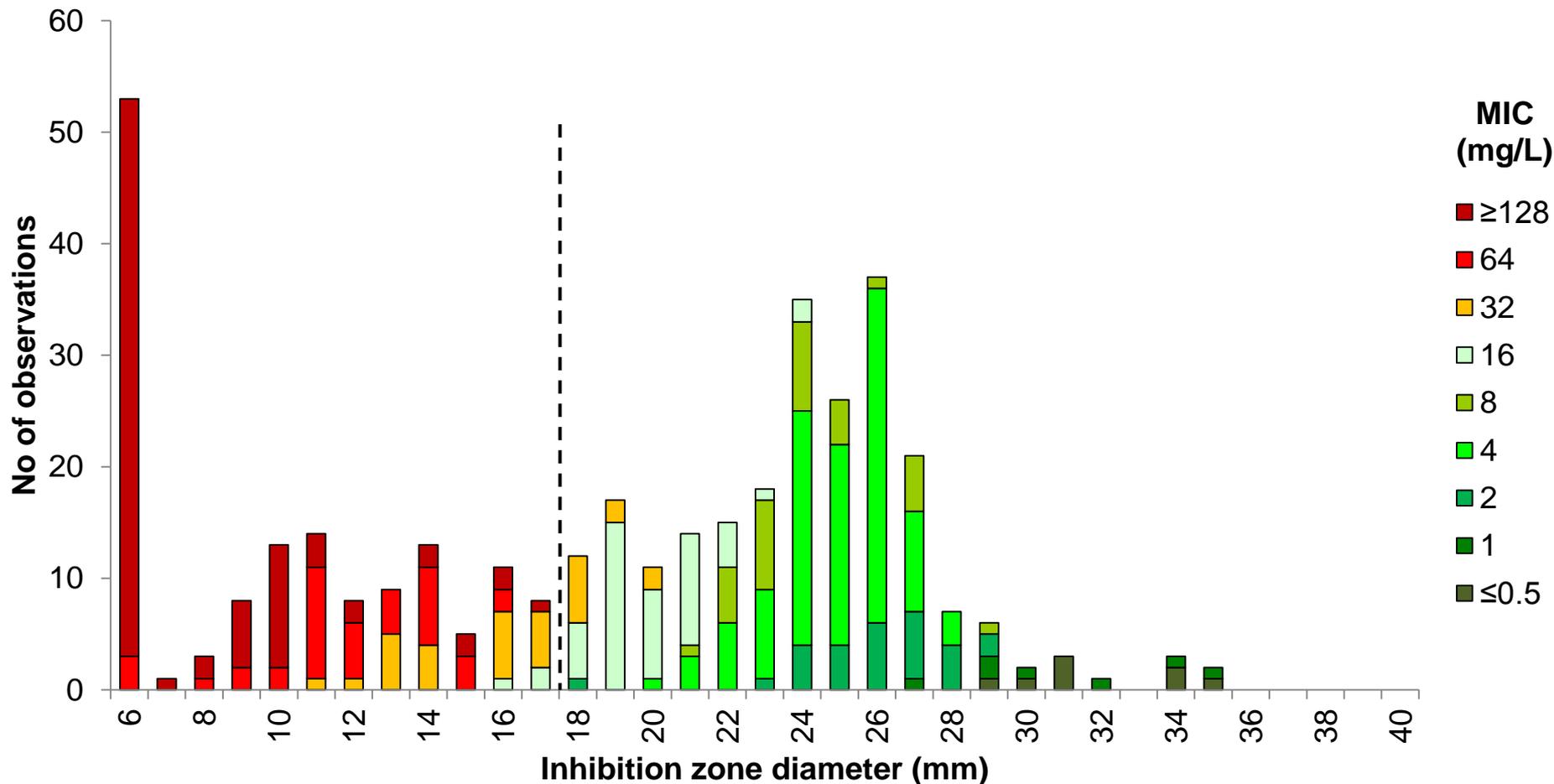
Piperacillin-tazobactam 30-6 μg vs. MIC *E. coli*, 294 isolates (496 correlates)



Breakpoints		ECOFF
MIC	S ≤ 8, R > 16 mg/L	8 mg/L
Zone diameter	S ≥ 20, R < 17 mm	

Piperacillin-tazobactam 30-6 μ g vs. MIC

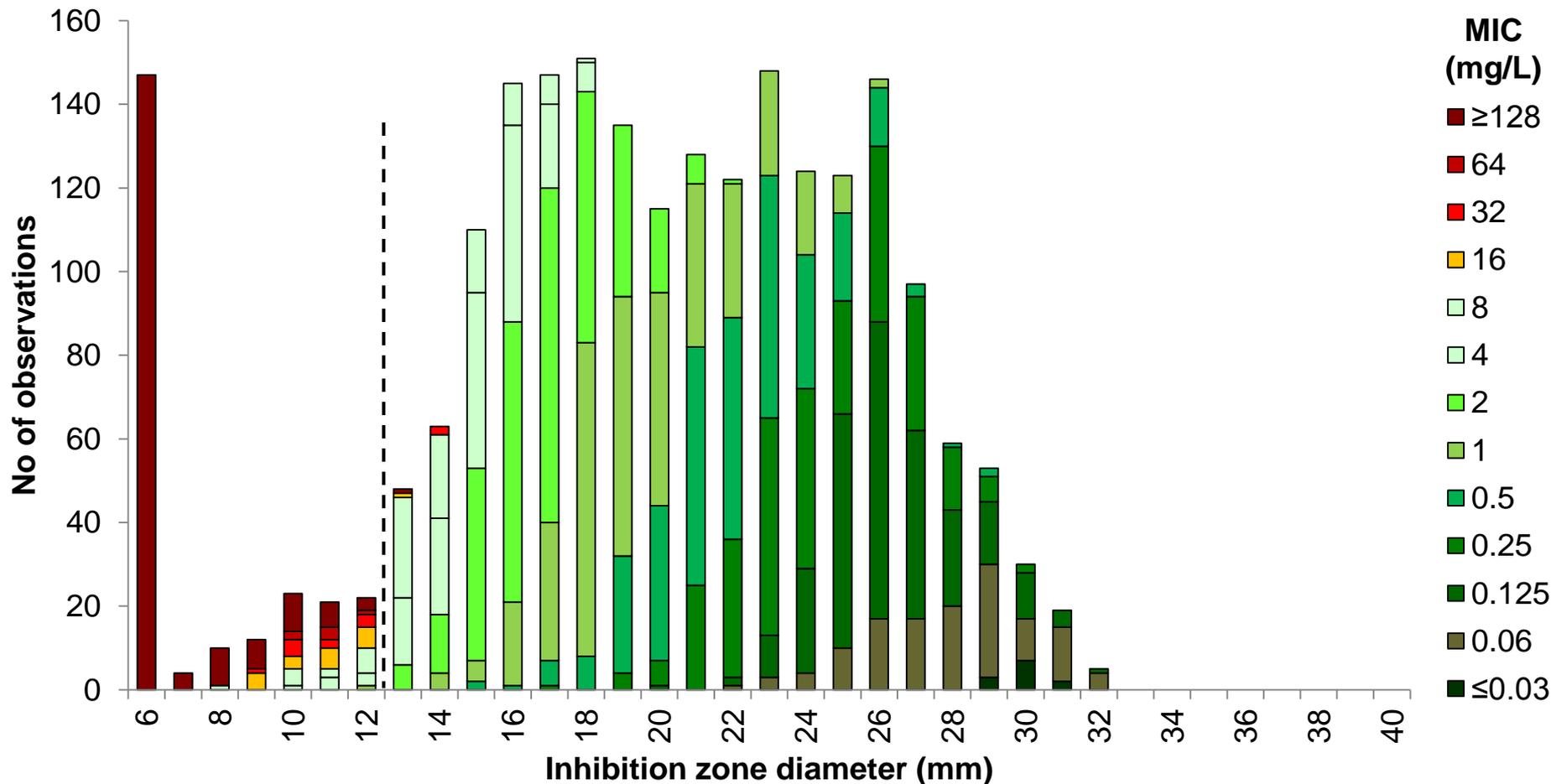
Pseudomonas aeruginosa, 217 isolates (376 correlates)



Breakpoints		ECOFF
MIC	$S \leq 16$, $R > 16$ mg/L	16 mg/L
Zone diameter	$S \geq 18$, $R < 18$ mm	

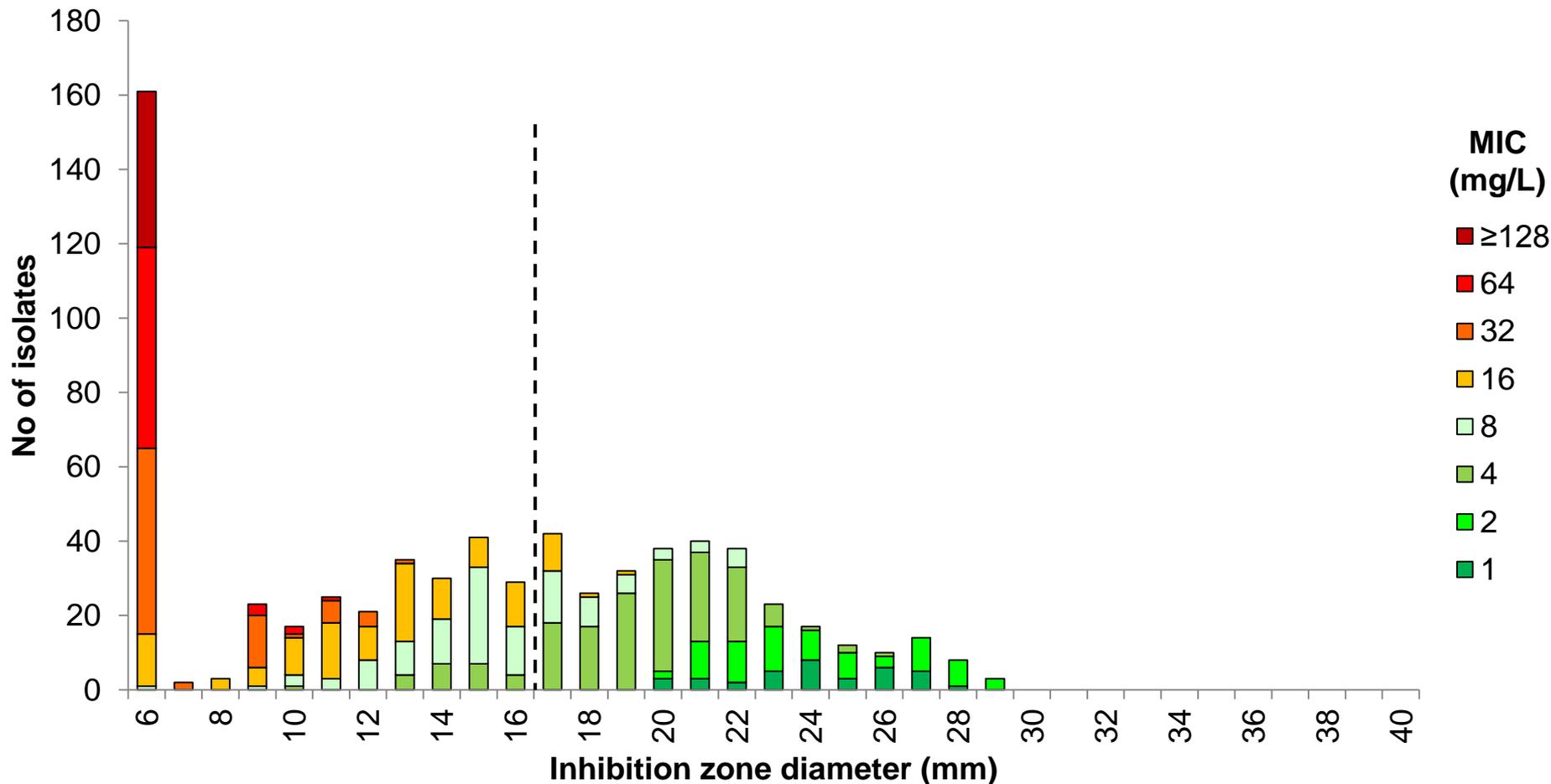
Ceftazidime-avibactam 10-4 µg vs. MIC

Enterobacteriaceae, 293 isolates (2207 correlates)



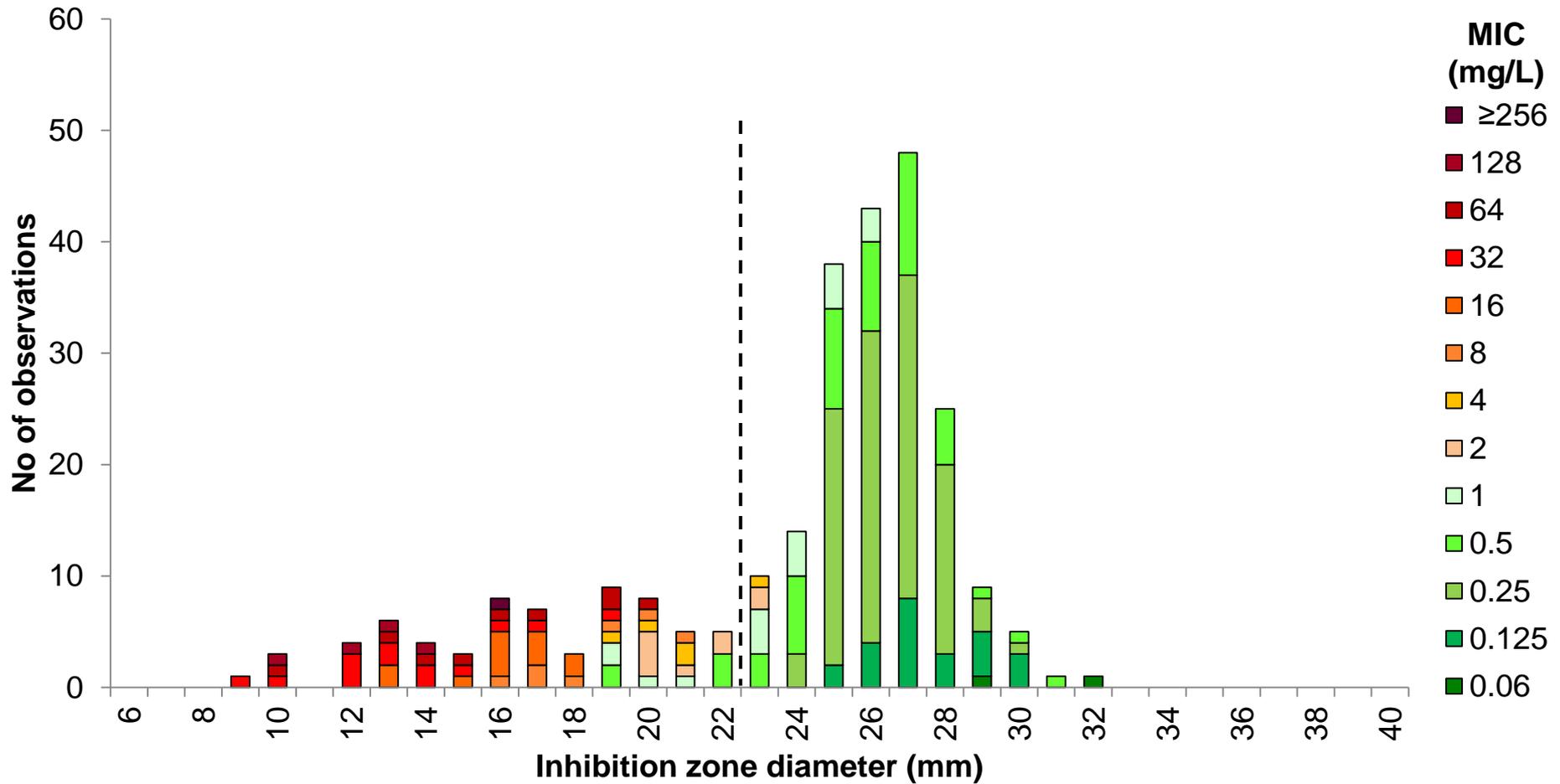
Ceftazidime-avibactam 10-4 µg vs. MIC

Pseudomonas aeruginosa, 100 isolates (690 correlates)



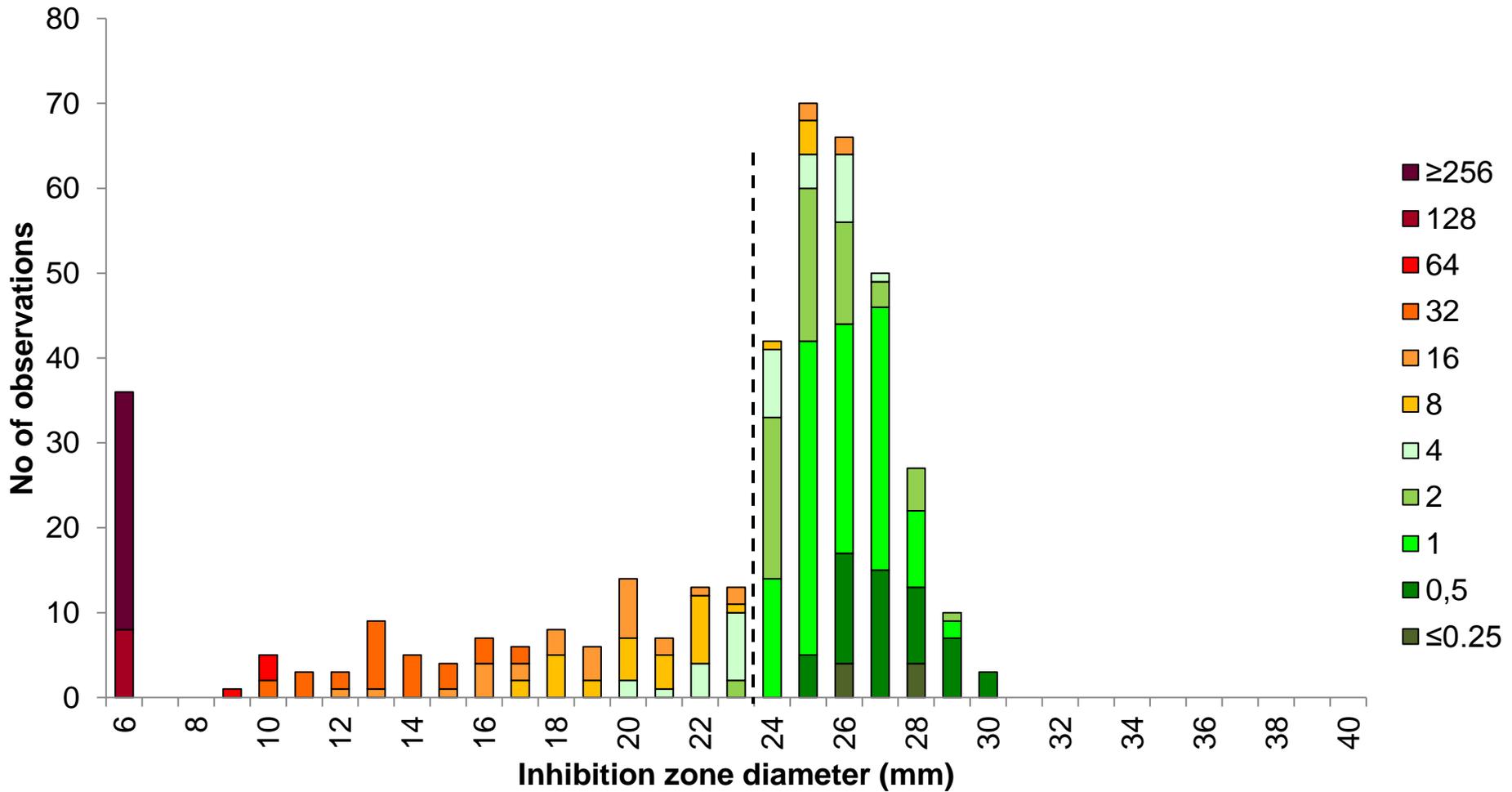
Breakpoints	ECOFF
MIC	8 mg/L
Zone diameter	8 mg/L

Ceftolozane-tazobactam 30-10 µg vs. MIC Enterobacteriaceae, 260 isolates



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

Ceftolozane-tazobactam 30-10 µg vs. MIC *P. aeruginosa*, 102 isolates (408 correlates)



Breakpoints	
MIC	S \leq 4, R $>$ 4 mg/L
Zone diameter	S \geq 24, R $<$ 24 mm

