

How are zone diameter breakpoints set and how are they related to MIC breakpoints?

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Reference MIC values

Reference antimicrobial susceptibility testing

- Broth microdilution (BMD) MIC determination is the international reference method for antimicrobial susceptibility testing (ISO 20776-1).
- Standardisation of:
 - Potency of antimicrobial agent
 - Antimicrobial concentrations (doubling dilutions)
 - Medium
 - Inoculum
 - Incubation
 - Reading of results

Gold standard = MIC determination according to ISO 20776-1

MIC = Minimum Inhibitory Concentration

“The lowest concentration that, under defined in vitro conditions, prevents visible growth of bacteria within a defined period of time”

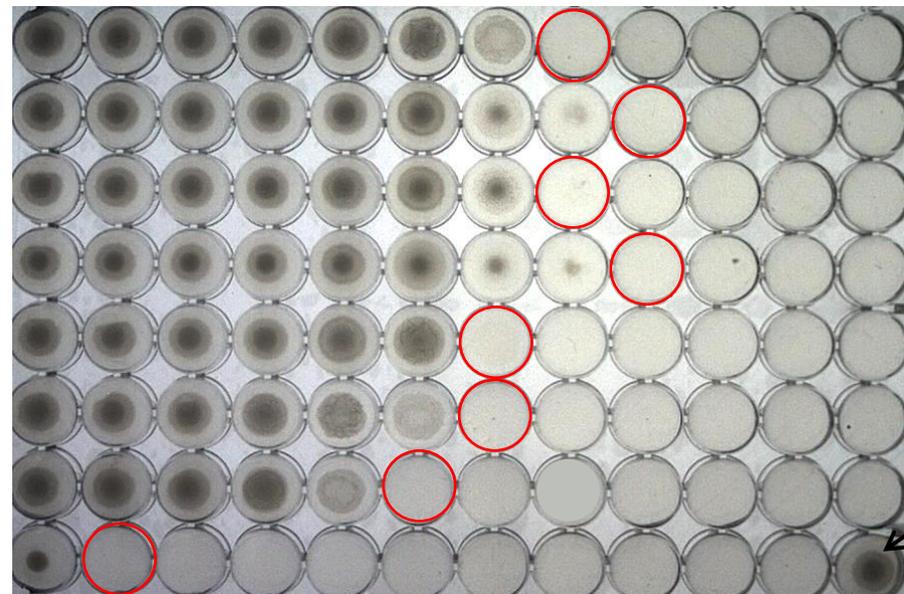
INTERNATIONAL
STANDARD

ISO
20776-1

Second edition
2019-06

Susceptibility testing of infectious
agents and evaluation of performance
of antimicrobial susceptibility test
devices —

Part 1:
Broth micro-dilution reference
method for testing the in vitro activity
of antimicrobial agents against rapidly
growing aerobic bacteria involved in
infectious diseases



Antimicrobial concentration →

Positive control

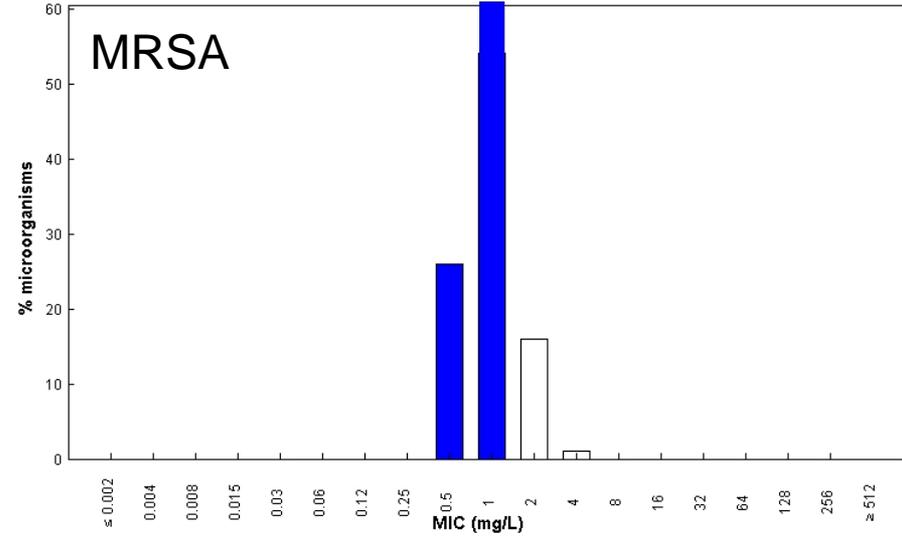
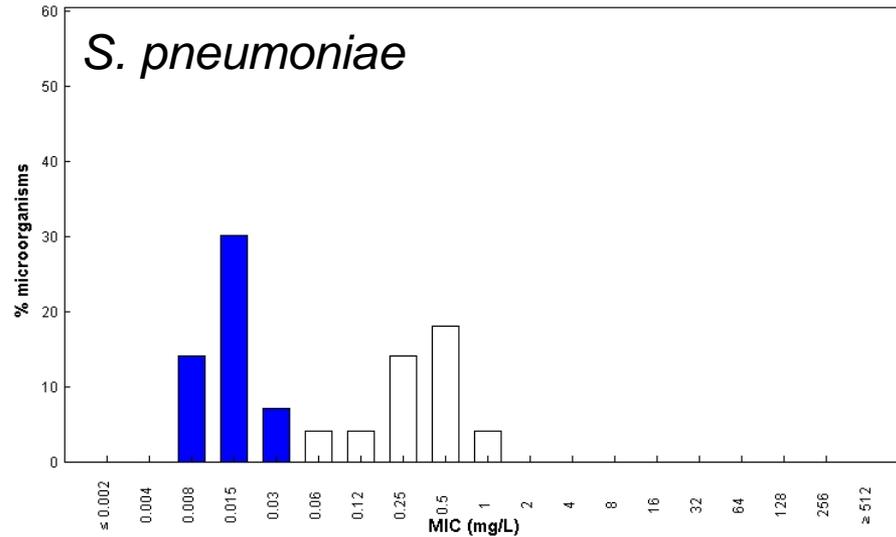
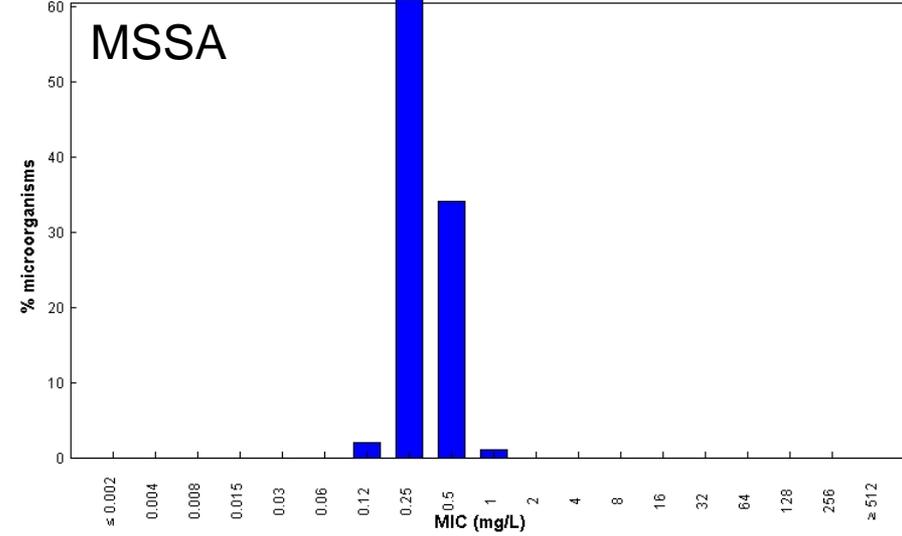
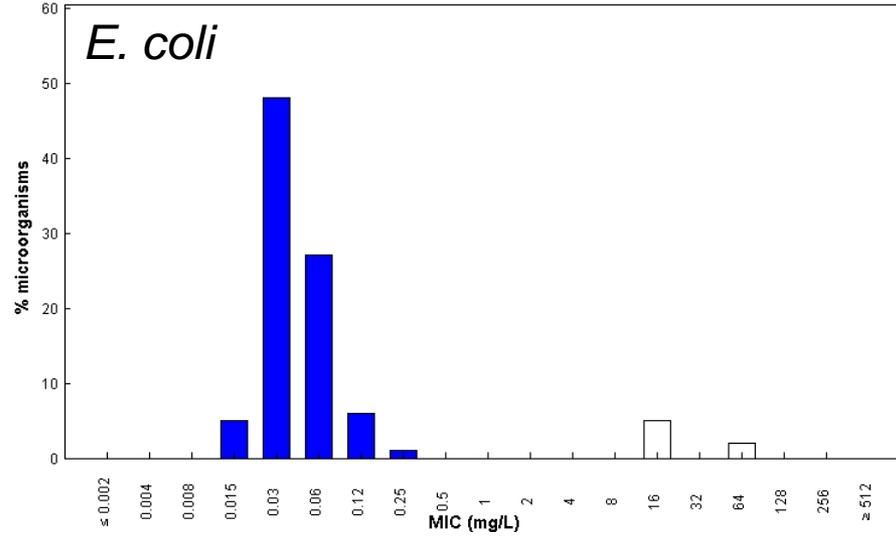
Development of MIC methodology for a specific agent

- MIC distributions are an important part of the process to establish clinical breakpoints
- The MIC reference methodology for the antimicrobial agent must be defined before generating MIC distributions
 - Special test situations/supplements
 - Specific reading instructions
 - Agent-inhibitor combinations
 - Ratio or fixed concentration

Microbiological activity

- MIC distributions for relevant Gram-negative and Gram-positive bacteria
 - Define target species
 - Identify wild-type isolates
 - Identify isolates with phenotypic resistance

Example ceftobiprole



Clinical MIC breakpoints

Why should you bother?

Enterobacteriaceae 1975 – 2001

Committee	Amoxicillin	Cefotaxime	Piperacillin-tazob.
BSAC (UK)	8 / 16	2 / 2	16 / 16
CA-SFM (F)	4 / 16	4 / 32	8 / 64
CRG (NL)	2 / 16	4 / 8	0.25 / 4
DIN (D)	2 / 8	2 / 8	0.12 / 1
NCCLS (USA)	8 / 16	8 / 32	16 / 64
NWGA (N)	0.5 / 8	1 / 2	8 / 16
SRGA (S)	1 / 8	0.5 / 1	16 / 16

Who are the stakeholders?

EUCAST* (Christian Giske)	EMA, Europe, ++
CLSI (Mel Weinstein)	USA, ++
FDA	USA
Colleagues who know better	Everywhere

*Organizing the national committees in Europe and elsewhere
(USCAST, CanCAST, AusNAC, BrCAST, ChiCAST)

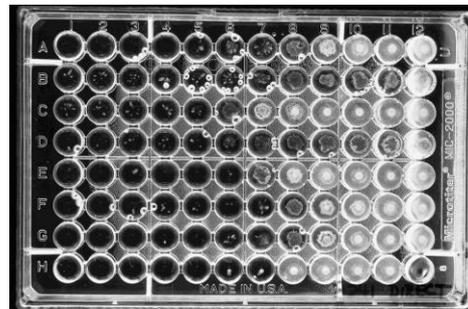
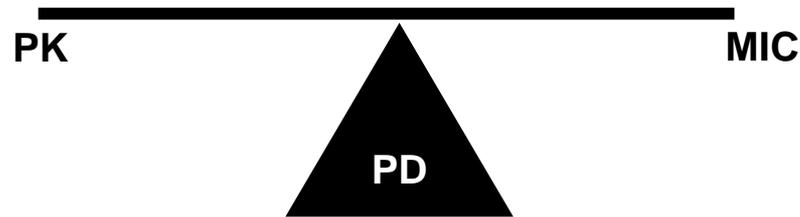
The process of setting breakpoints



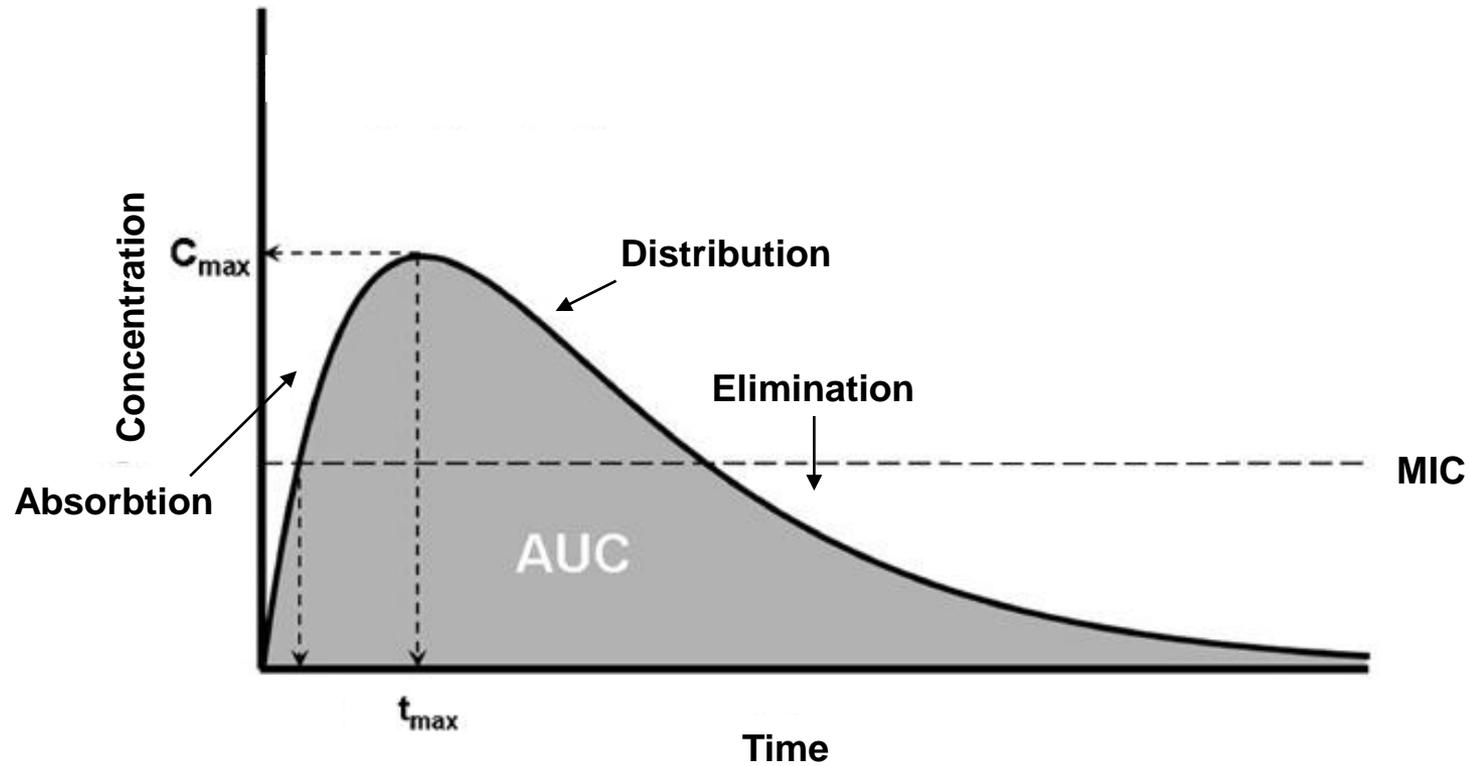
The EUCAST internal process

- Companies are not allowed to take part of the closed discussions
- Detailed minutes of the discussion will be shared
- Iterative process of comments and responses, which can go on for several months, and overlap with EMA's LoQ
- Ultimately, EUCAST makes a decision
- EUCAST does not conduct any voting
- When the agent has been approved by EMA, breakpoints will be published on the EUCAST website
- A rationale document will be published on the EUCAST website

Killing of bacteria – PK/PD



AUC and $T > MIC$



The targets

Antimicrobial	PD parameter	Target
Penicillins	%fT>MIC	50
Cephalosporins	%fT>MIC	50
Carbapenems	%fT>MIC	40
Fluoroquinolones	fAUC/MIC	Gram-positive: 40 Gram-negative: 80
Aminoglycosides	AUC/MIC	30-40
Tigecycline	AUC/MIC	Gram-positive: 12.5 Gram-negative: 7
Vancomycin	fAUC/MIC	180 (<i>S. aureus</i>)

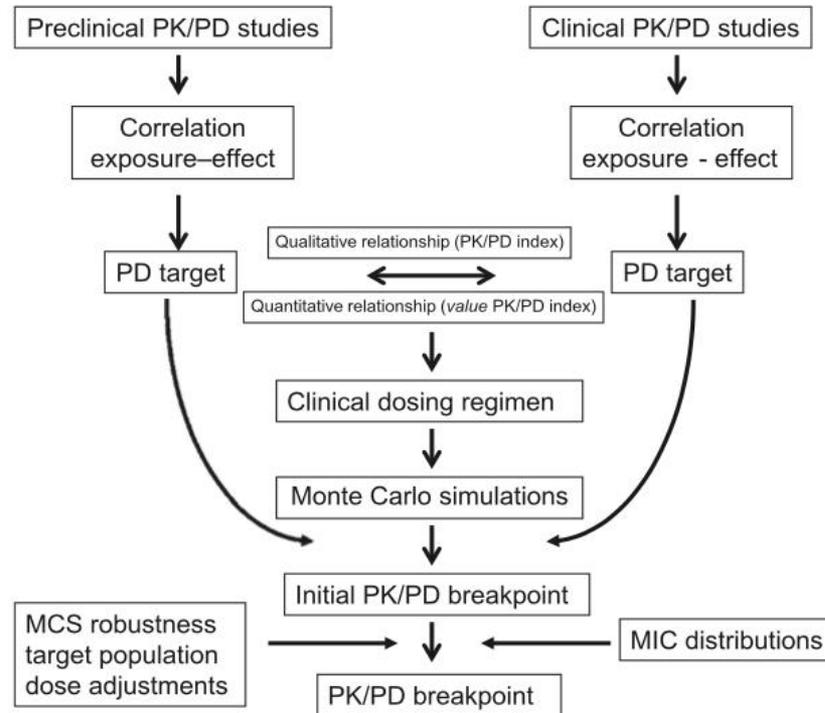
Which drug has highest activity

- Antimicrobial A: MIC 0.5 mg/L
 - Antimicrobial B: MIC 8 mg/L
-
- Antimicrobial A: Area under serum concentration curve (AUC): 5
 - Antimicrobial B: AUC 120
-
- Antimicrobial A: $AUC/MIC=5/0.5=10$
 - Antimicrobial B: $AUC/MIC=120/8=15$

Overview of the process

- Clinical data (exposure vs outcome)
- PK-PD-data
 - Generally, 95-99% target attainment is required
- Wild-type distribution assessment (definition of ECOFF)
 - Avoid splitting the wild-type
- Information on resistance mechanisms
 - Clinical significance and MICs for organisms with resistance mechanisms
- Clinical indications: which species are relevant
- Established breakpoints can be reviewed
 - On the initiative of EUCAST, EMA or the pharmaceutical company

The process in graphics



Mouton JW et al. Clin Microbiol Infect 2012; 18: E37–E45

EUCAST SOPs for breakpoints

EUCAST controlled document	EUCAST SOP 1.2
Date of issue: 21 November 2016	Page 1 of 15



EUCAST EUROPEAN COMMITTEE
ON ANTIMICROBIAL
SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Standard Operating Procedure

Setting breakpoints for new antimicrobial agents

EUCAST SOP 1.2

21 November 2016

Setting breakpoints for new antimicrobial agents
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EUCAST controlled document	EUCAST SOP 2.2
Date of issue: 21 November 2016	Page 1 of 14



EUCAST EUROPEAN COMMITTEE
ON ANTIMICROBIAL
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Standard Operating Procedure

Harmonization of breakpoints for existing antimicrobial agents

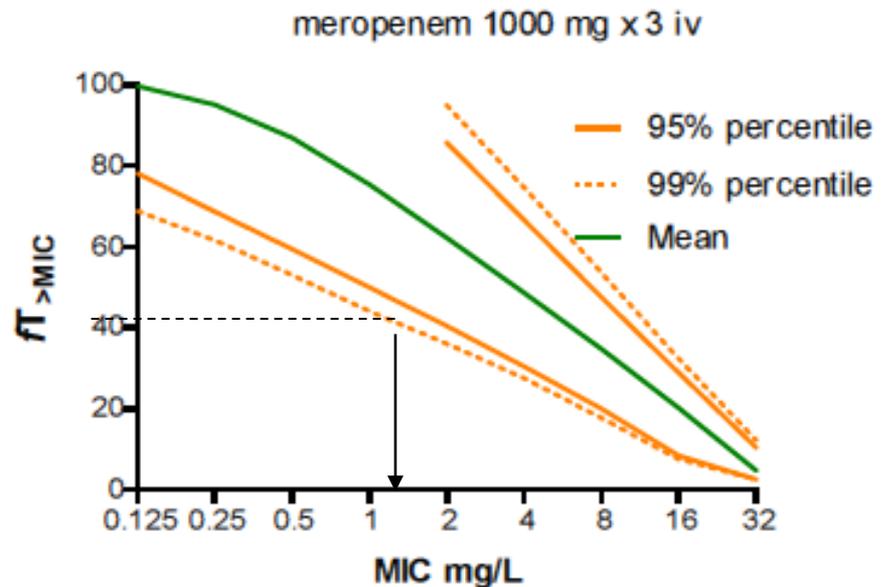
EUCAST SOP 2.2

21 November 2016

Harmonization of breakpoints for existing antimicrobial agents
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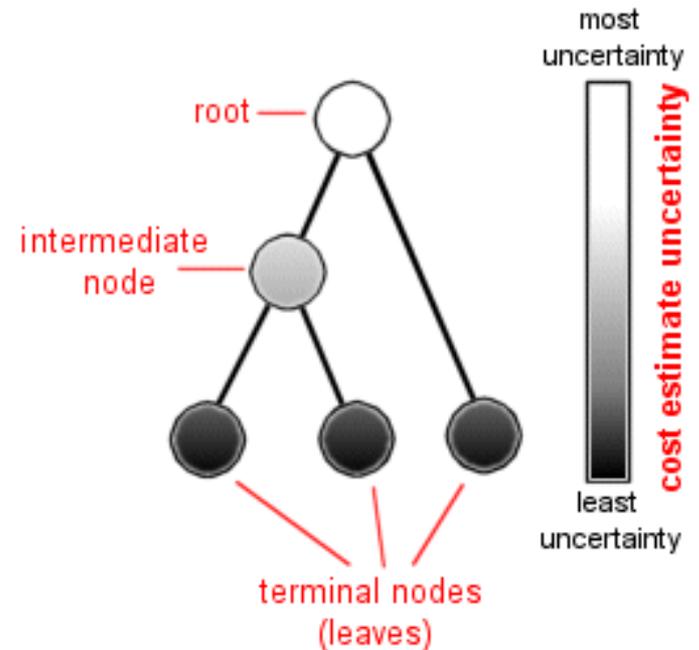
Monte Carlo simulations

- PK-data from relevant patient populations and healthy volunteers are used
- Mathematical simulation to increase variability in the dataset
- Calculate probability of reaching a predefined target (e.g. $T > MIC$)
- 95% of the population should reach the target
- Always applies for one specific dosing regimen



Assessing clinical data

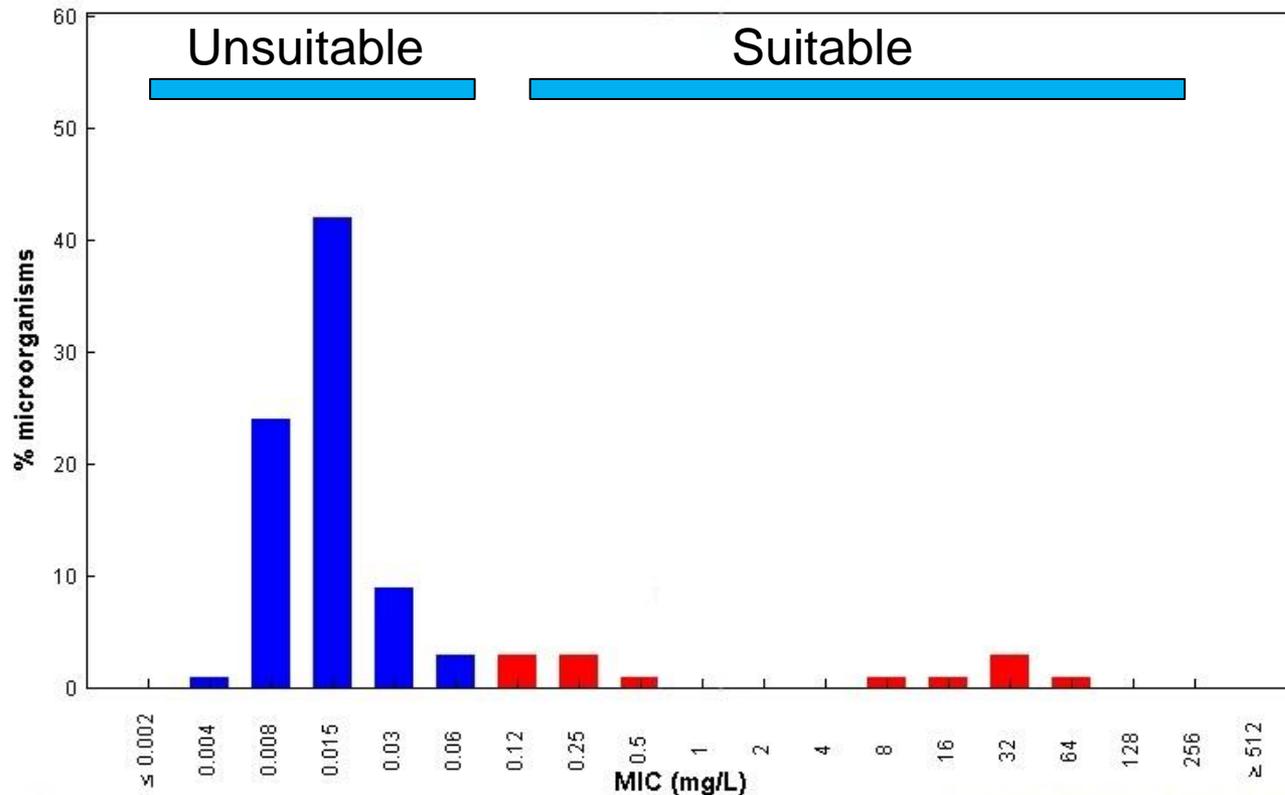
- Classification And Regression Tree
- Define individual AUC/MIC or $T > MIC$ from clinical trial
- Start iterative splitting in success and therapeutic failure
- Identify the node (value) that gives the best separation
- The node is a CART-based breakpoint
- A simplified variant is MIC vs outcome (not considering drug exposure)



The breakpoint should not split the wild type

Ciprofloxacin / *Escherichia coli*
EUCAST MIC Distribution - Reference Database 2011-11-01

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off: WT ≤ 0.064 mg/L

17877 observations (82 data sources)
Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L

SIR-definitions from EUCAST

- S - Susceptible, standard dosing regimen: A microorganism is categorised as "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.
- I - Susceptible, increased exposure*: A microorganism is categorised as "Susceptible, Increased exposure*" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.
- R - Resistant: A microorganism is categorised as "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure.

*Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution and excretion of the antimicrobial agent will influence the infecting organism at the site of infection.

Breakpoint tables

European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters

Version 10.0, valid from 2020-01-01

This document should be cited as "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. <http://www.eucast.org>."

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Viridans group streptococci	55	
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Moraxella catarrhalis	66	
Neisseria gonorrhoeae	70	

Nordic Committee on Antimicrobial Susceptibility Testing

Brytpunktstabeller för tolkning av MIC-värden och zondiametrar

NordicAST Version 10.0, gäller från 2020-02-07 [Baserat på EUCAST Version 10.0]

Innehåll	Sida	Externa länkar
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Enterobacterales	20	
Pseudomonas spp.	29	
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Acinetobacter spp.	34	
Staphylococcus spp.	36	
Enterococcus spp.	44	
Streptococcus groups A, B, C and G	48	
Streptococcus pneumoniae	53	
Viridans group streptococci	58	
Haemophilus influenzae	62	
Moraxella catarrhalis	67	
Neisseria gonorrhoeae	70	

All breakpoints are dose-related

Dosages

EUCAST Clinical Breakpoint Tables v. 10.0, valid from 2020-01-01

EUCAST breakpoints are based on the following dosages (see section 8 in Rationale Documents). Alternative dosing regimens which result in equivalent exposure are acceptable. The table should not be considered an exhaustive guidance for dosing in clinical practice, and does not replace specific local, national, or regional dosing guidelines. However, if national practices significantly differ from those listed below, EUCAST breakpoints may not be valid. Situations where less antibiotic is given as standard or high dose should be discussed locally or regionally.

Uncomplicated UTI: acute, sporadic or recurrent lower urinary tract infections (uncomplicated cystitis) in patients with no known relevant anatomical or functional abnormalities within the urinary tract or comorbidities.

Penicillins	Standard dose	High dose	Uncomplicated UTI	Special situations
Benzylpenicillin	0.6 g (1 MU) x 4 iv	1.2 g (2 MU) x 4-6 iv		Meningitis caused by <i>S. pneumoniae</i> : For a dose of 2.4 g (4 MU) x 6 iv, isolates with MIC \leq 0.06 mg/L are susceptible. Pneumonia caused by <i>S. pneumoniae</i>: breakpoints are related to dosage: For a dose of 1.2 g (2 MU) x 4 iv, isolates with MIC \leq 0.5 mg/L are susceptible. For a dose of 2.4 (4 MU) g x 4 iv or 1.2 g (2 MU) x 6 iv, isolates with MIC \leq 1 mg/L are susceptible. For a dose of 2.4 g (4 MU) x 6 iv, isolates with MIC \leq 2 mg/L are susceptible.
Ampicillin	2 g x 3 iv	2 g x 4 iv		Meningitis: 2 g x 6 iv
Ampicillin-sulbactam	(2 g ampicillin + 1 g sulbactam) x 3 iv	(2 g ampicillin + 1 g sulbactam) x 4 iv		
Amoxicillin iv	1 g x 3-4 iv	2 g x 6 iv		Meningitis: 2 g x 6 iv
Amoxicillin oral	0.5 g x 3 oral	0.75-1 g x 3 oral	0.5 g x 3 oral	H. influenzae: High-dose-only
Amoxicillin-clavulanic acid iv	(1 g amoxicillin + 0.2 g clavulanic acid) x 3-4 iv	(2 g amoxicillin + 0.2 g clavulanic acid) x 3 iv		
Amoxicillin-clavulanic acid oral	(0.5 g amoxicillin + 0.125 g clavulanic acid) x 3 oral	(0.875 g amoxicillin + 0.125 g clavulanic acid) x 3 oral	(0.5 g amoxicillin + 0.125 g clavulanic acid) x 3 oral	Amoxicillin-clavulanic acid has separate breakpoints for systemic infections and uncomplicated UTI. When amoxicillin-clavulanic acid is reported for uncomplicated UTI, the report must make clear that the susceptibility category is only valid for uncomplicated UTI. H. influenzae : High-dose-only
Piperacillin	4 g x 3 iv	4 g x 4 iv		Pseudomonas spp.: High-dose-only
Piperacillin-tazobactam	(4 g piperacillin + 0.5 g tazobactam) x 3 iv	(4 g piperacillin + 0.5 g tazobactam) x 4 iv		Pseudomonas spp.: High-dose-only

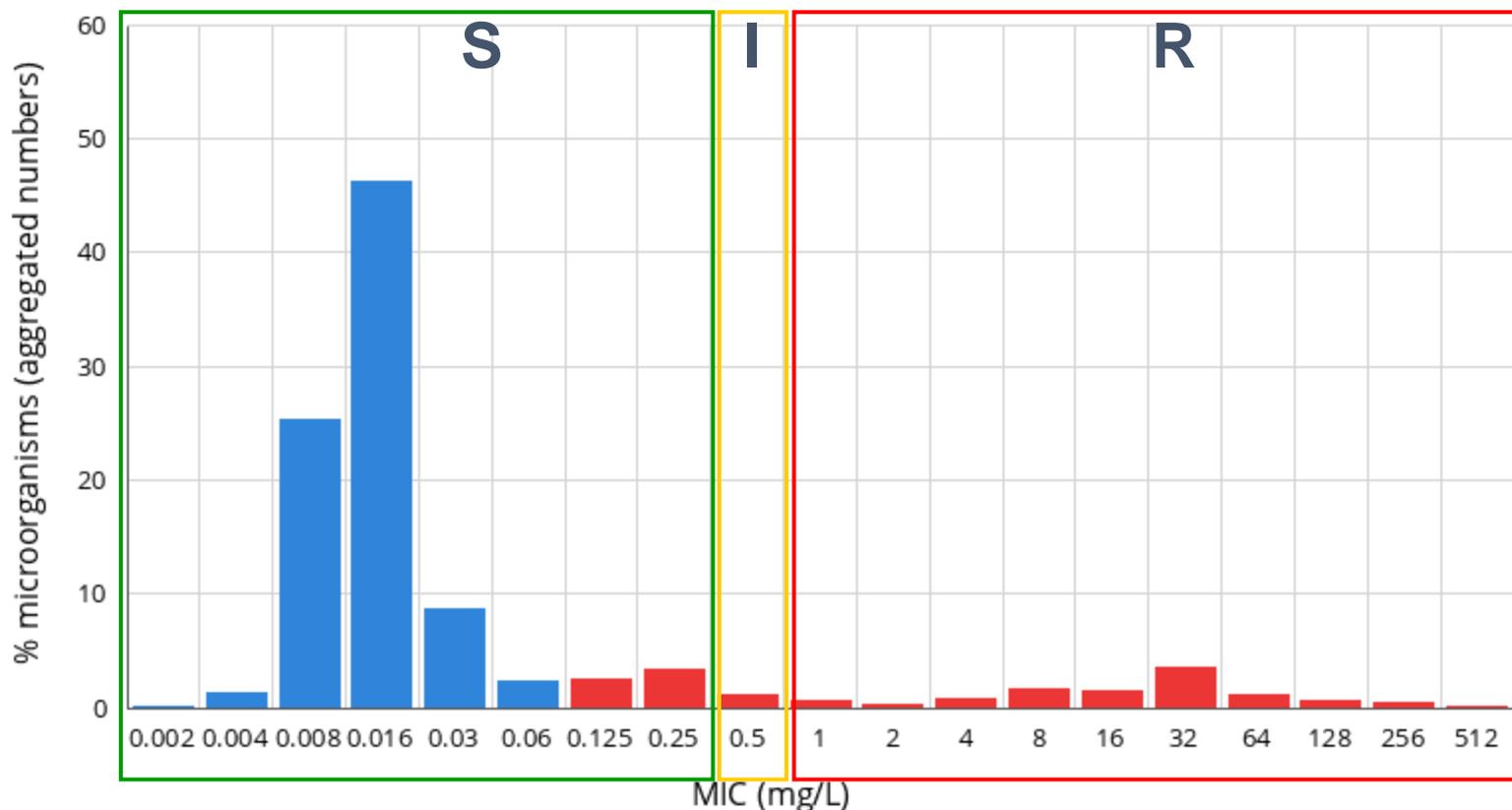
If another dose is used, the breakpoints are not applicable

Calibration of zone diameter breakpoints

$S \leq 0.25$, $R > 0.5$ mg/L

Ciprofloxacin / Escherichia coli
International MIC distribution - Reference database 2023-04-24
Based on aggregated distributions

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



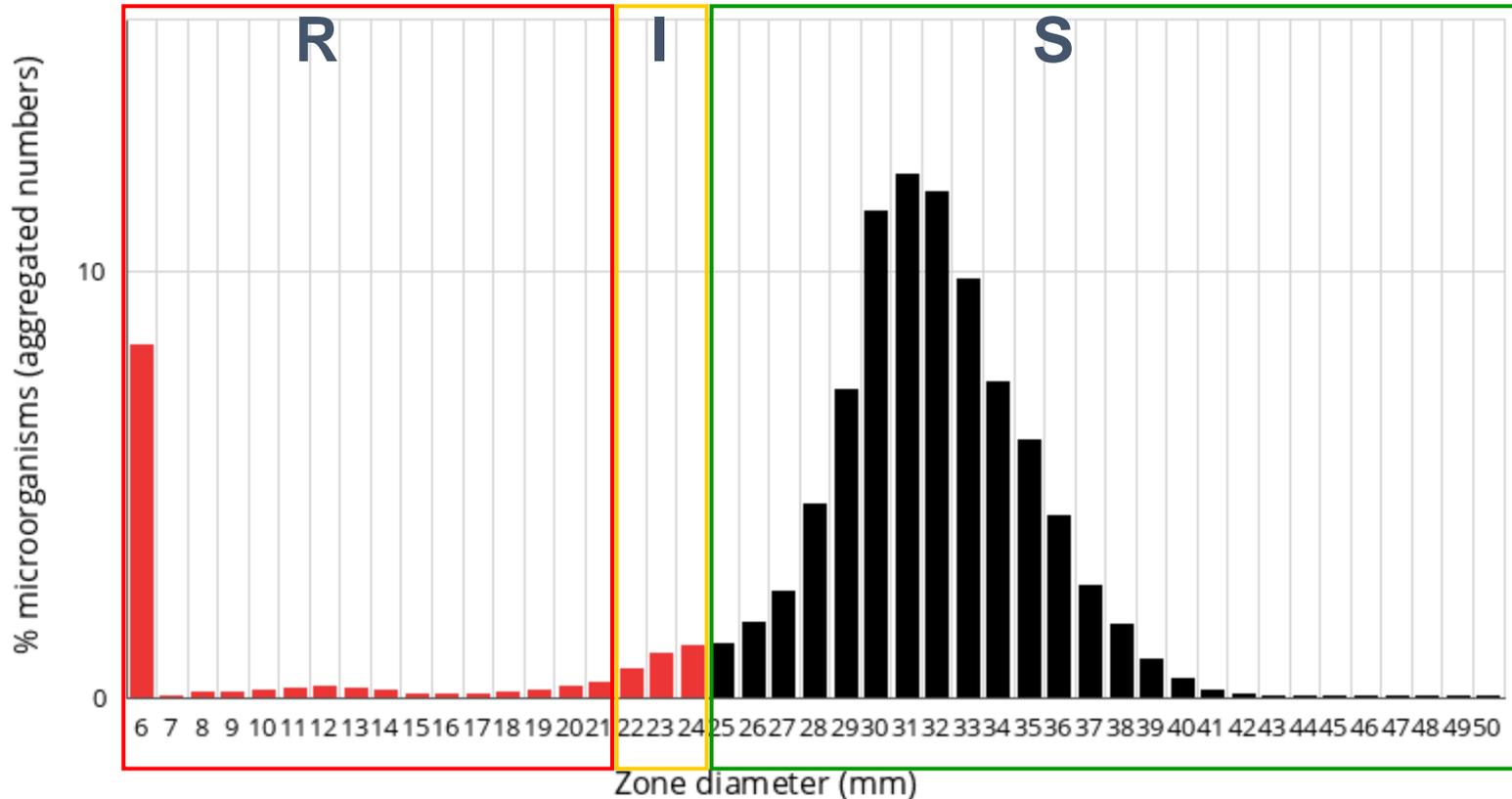
MIC
Epidemiological cut-off (ECOFF): 0.06 mg/L
Wildtype (WT) organisms: ≤ 0.06 mg/L

Confidence interval: 0.03 - 0.06
15667 observations (53 data sources)

S \geq 25, R<22 mm

Ciprofloxacin / Escherichia coli
International zone diameter distribution - Reference database 2023-04-24
EUCAST disk diffusion method
Based on aggregated distributions

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 (ECOFF): 25 mm
Wildtype (WT) organisms: \geq 25 mm

Confidence interval: -
57956 observations (16 data sources)

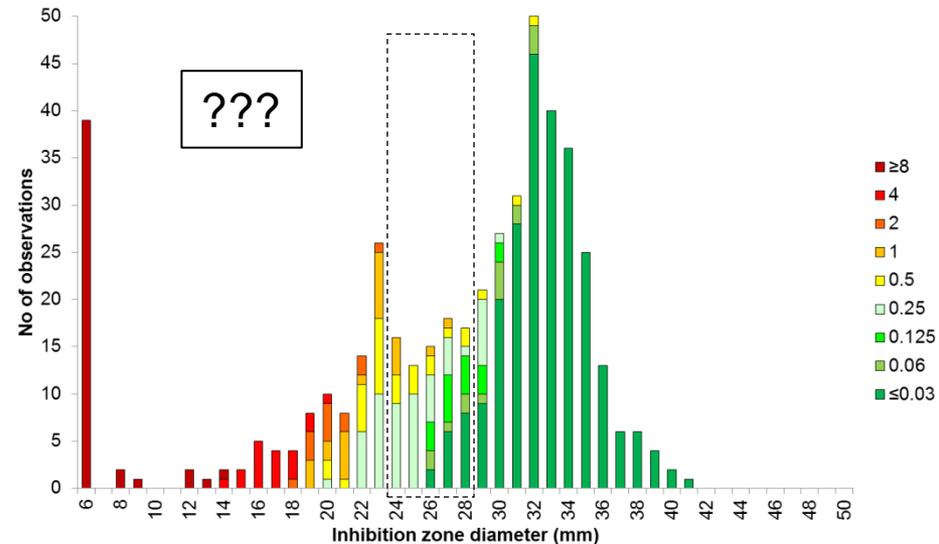
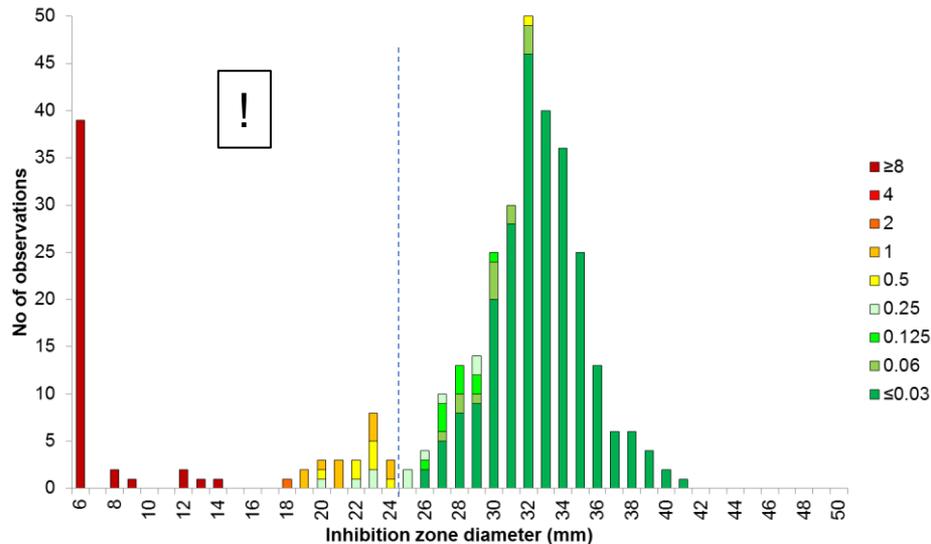
Calibration of disk diffusion to BMD MIC determination

- Parallel disk diffusion and BMD for clinical isolates representing WT isolates and isolates with elevated MICs.
- Correlation of inhibition zone diameters to BMD MICs (and sometimes resistance mechanism).
- Establishment of zone diameter breakpoints corresponding to EUCAST clinical MIC breakpoints.
 - Breakpoints are set to minimise false susceptibility.

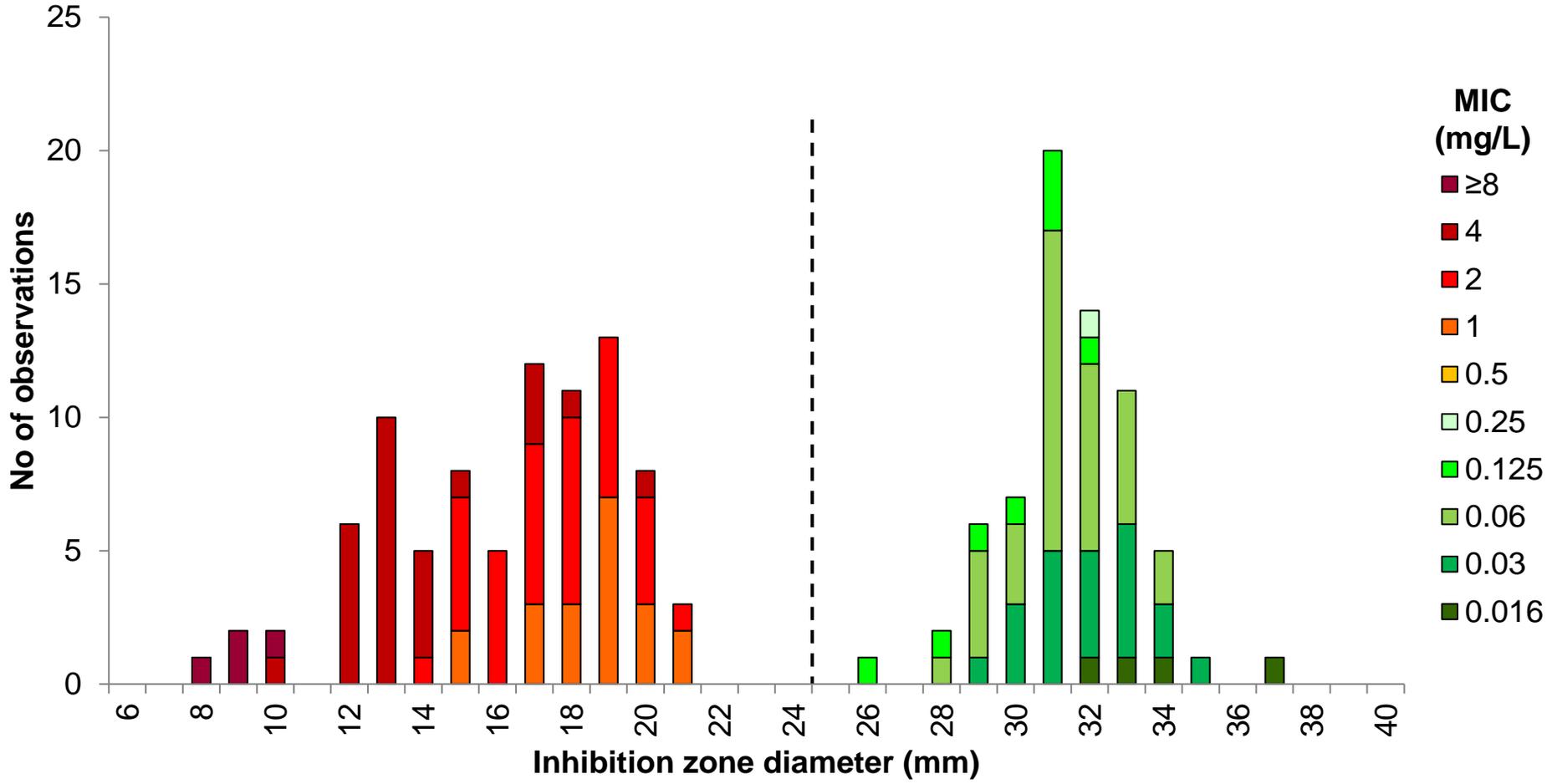
Isolates for MIC-zone diameter correlation studies

- The composition of the isolate collection greatly affects the results!

Breakpoints are set to minimise false susceptibility

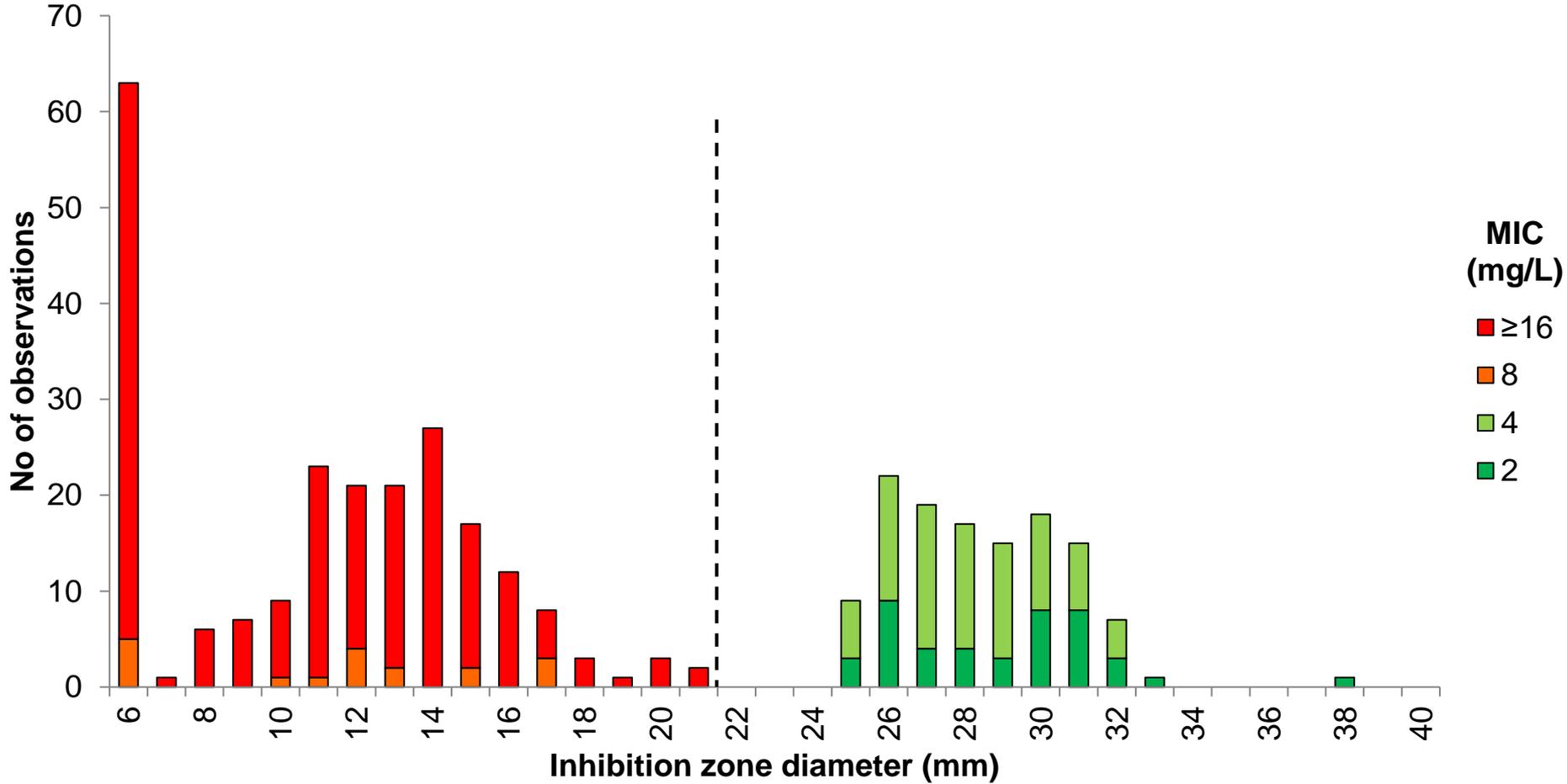


Moxifloxacin 5 μ g vs. MIC *S. aureus*, 154 isolates



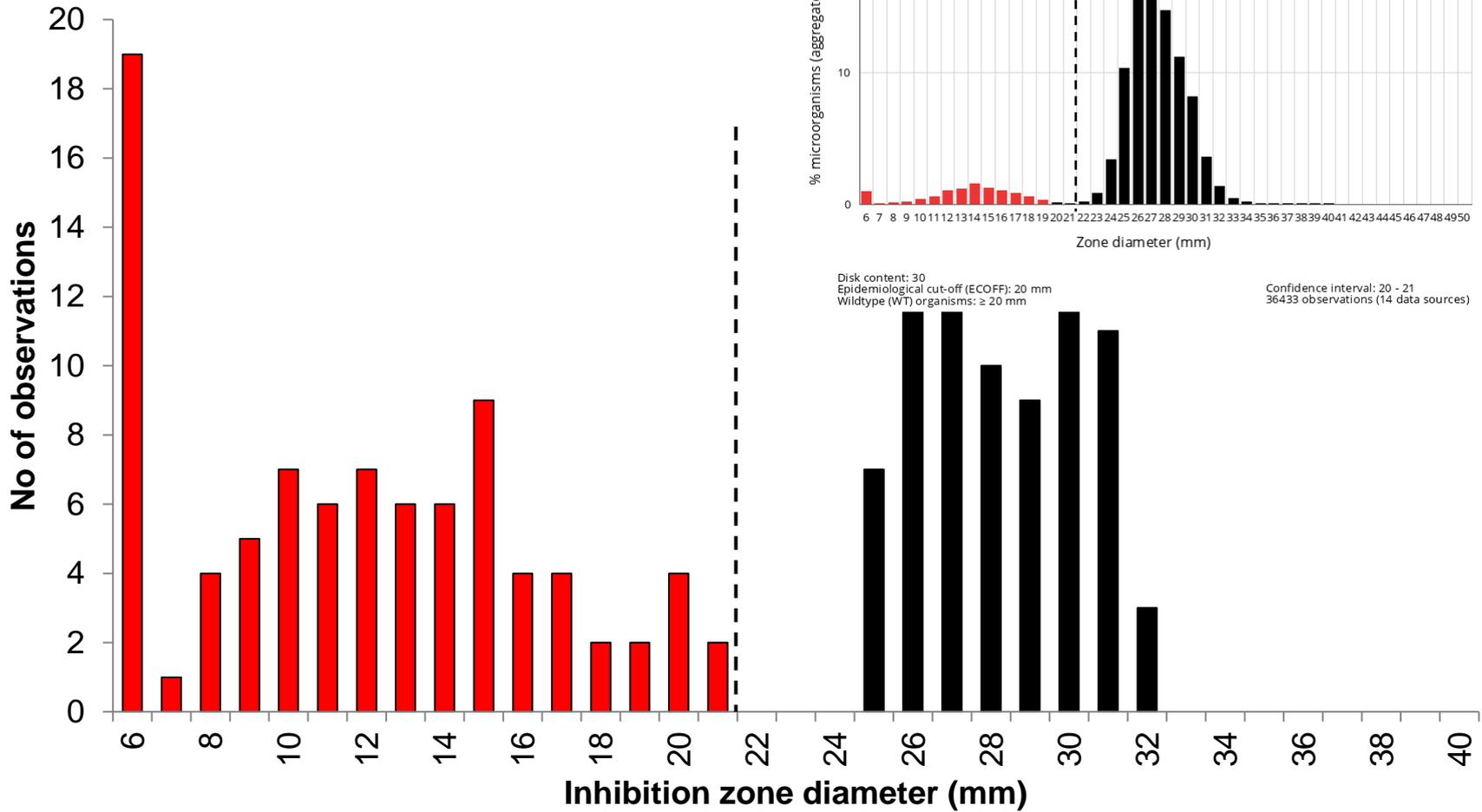
Cefoxitin 30 µg vs. MIC

S. aureus, 287 isolates (348 correlates)



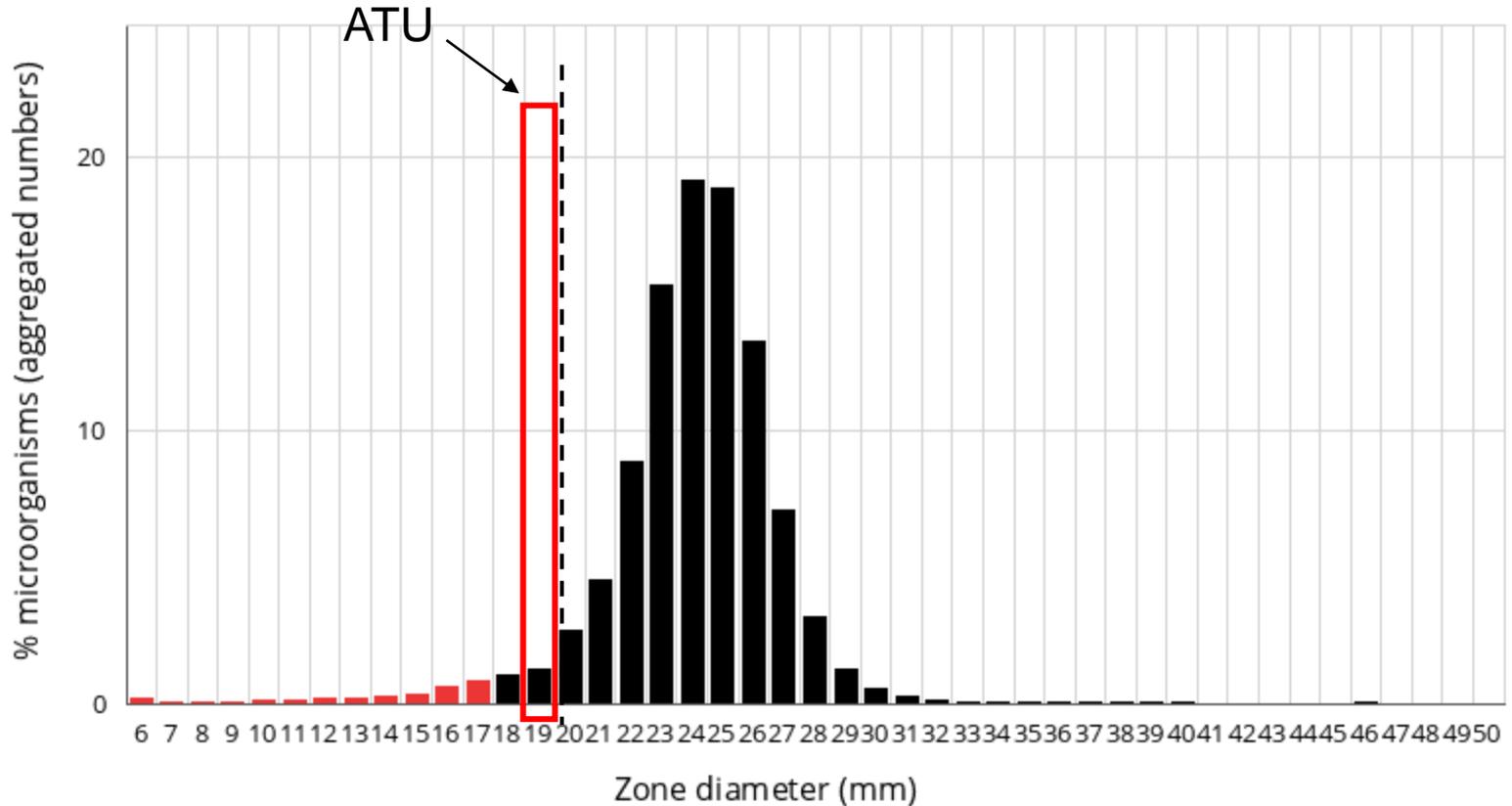
Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

Cefoxitin 30 µg *S. aureus*,



Piperacillin-tazobactam / Escherichia coli
International zone diameter distribution - Reference database 2023-04-24
EUCAST disk diffusion method
Based on aggregated distributions

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

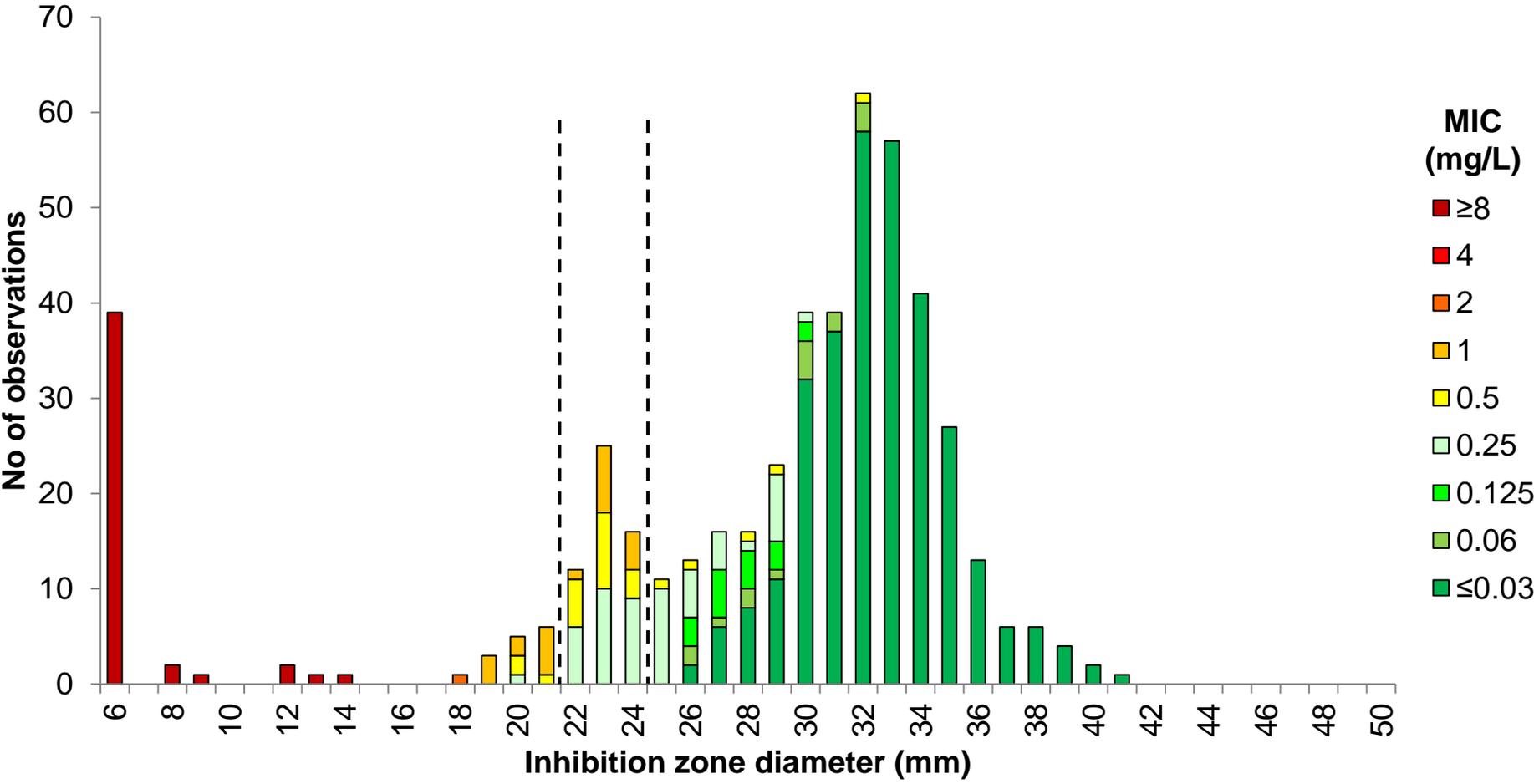


Disk content: 36
Epidemiological cut-off (ECOFF): 18 mm
Wildtype (WT) organisms: ≥ 18 mm

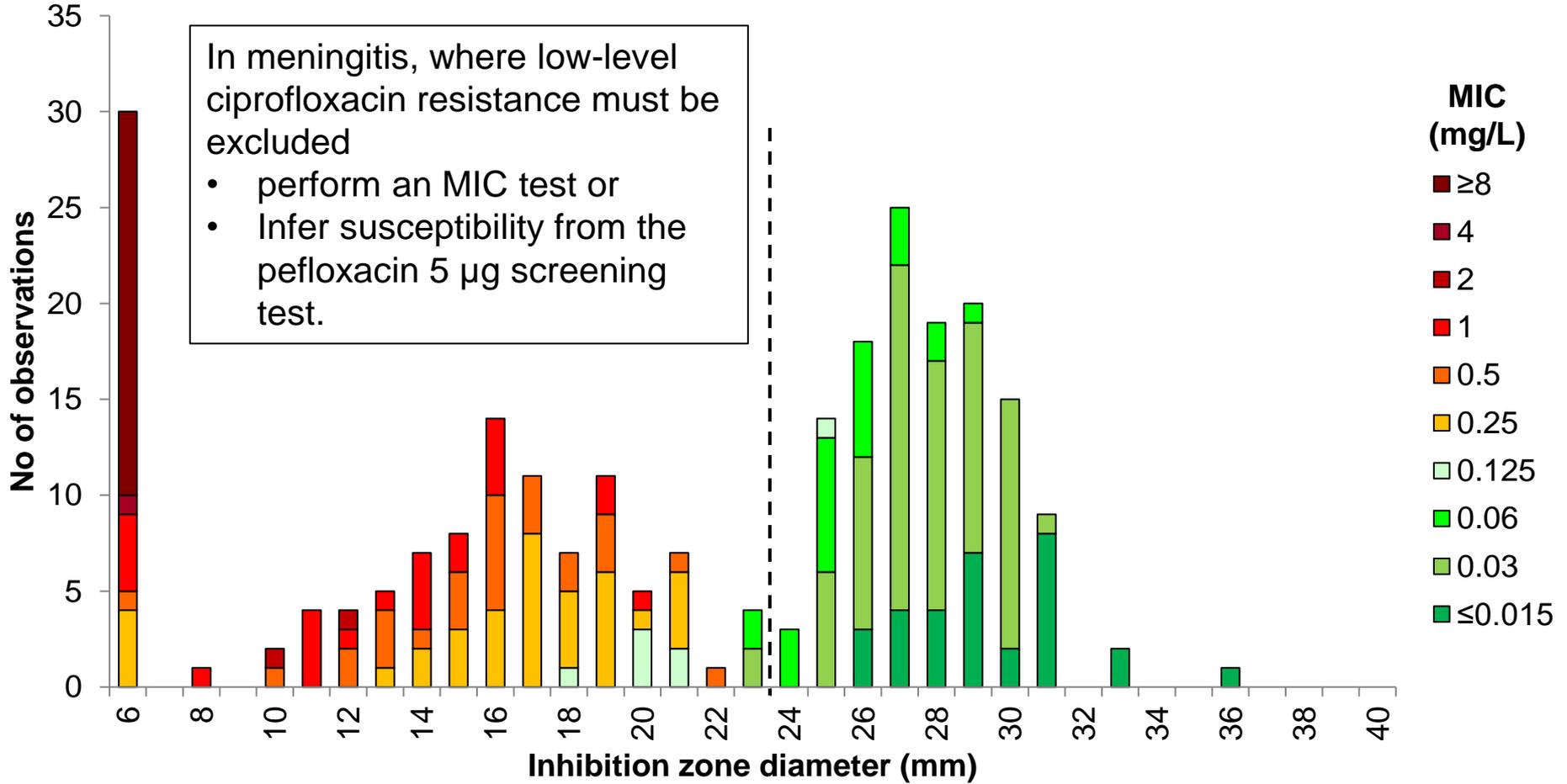
Confidence interval: 18 - 19
57404 observations (12 data sources)

Ciprofloxacin 5 μ g vs. MIC

Enterobacteriales, 471 isolates (490 correlates)



Pefloxacin 5 μ g vs. Ciprofloxacin MIC *Enterobacteriales*, 239 isolates (247 correlates)

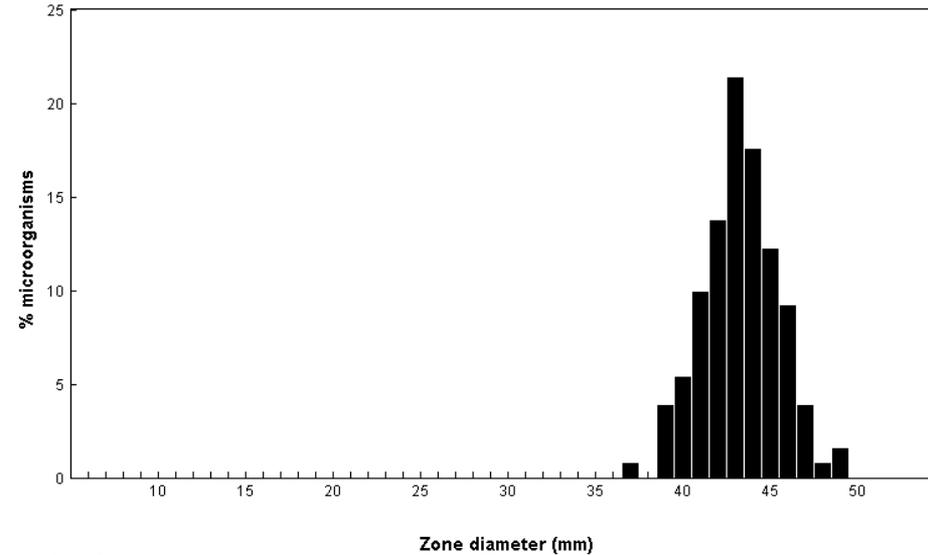
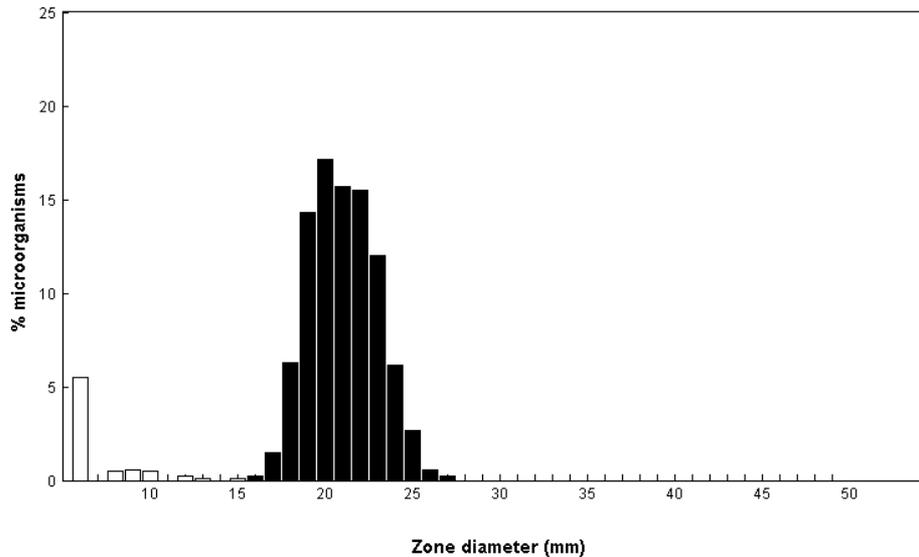




Selecting the optimal disk potency

Selecting disk potency

- Optimized inhibition zone size

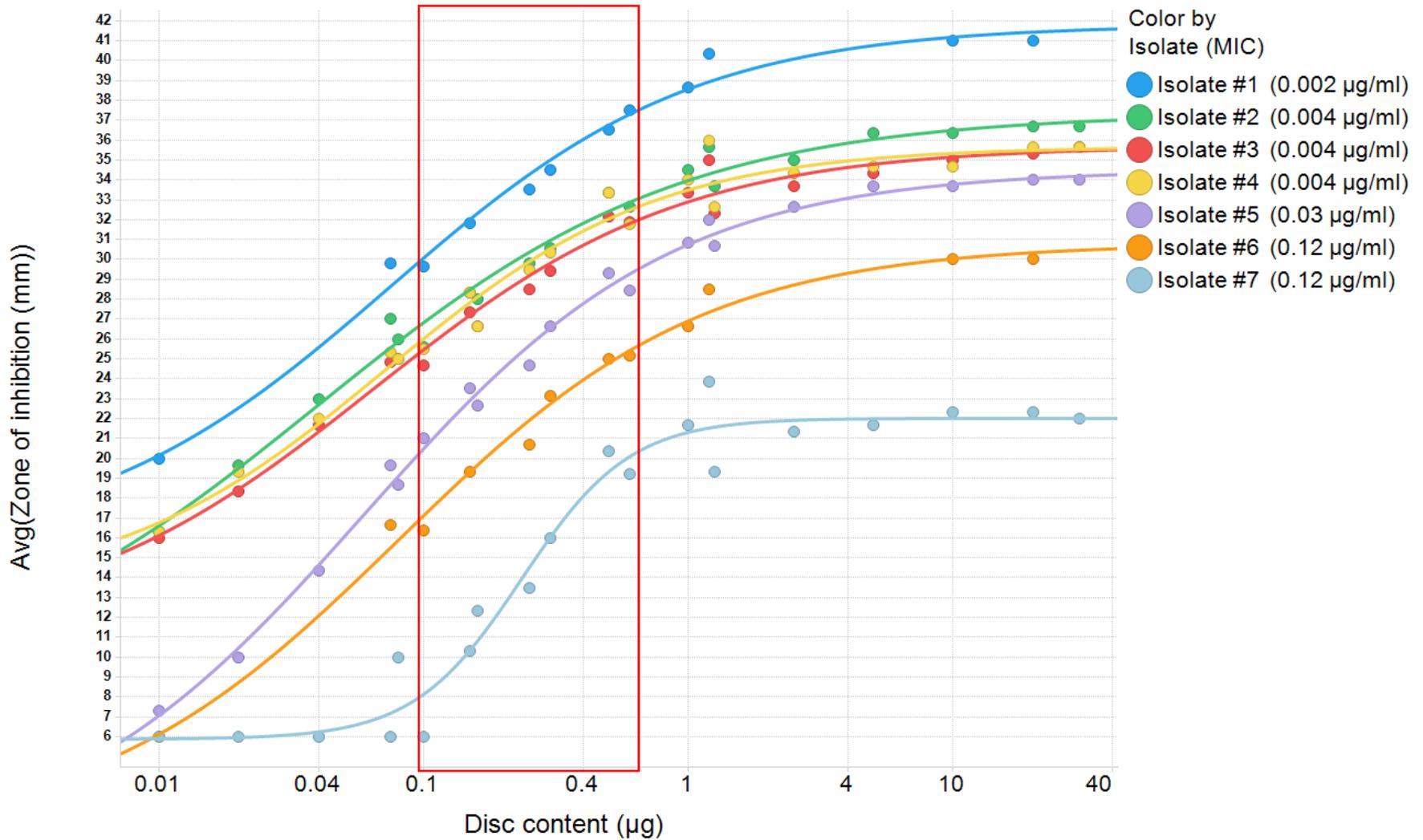


Selecting disk potency

- 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*

20 µg disk

