Application of EUCAST methods in Microbiology laboratory

Course attended: ESCMID Postgraduate Education Course: Antimicrobial susceptibility testing with EUCAST criteria and methods, Melbourne, Australia, 28 - 31 October 2023 organised by European Society of Clinical Microbiology and Infectious Diseases

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Clinical breakpoints and dosing of antibiotics

The European Committee on Antimicrobial Susceptibility Testing – EUCAST

Clinical breakpoints - breakpoints and guidance

January 2, 2023

- **Clinical breakpoints (v 14.0)** - file for printing (1 Jan, 2024)
- **Clinical breakpoints (v 14.0)** - file for screen (1 Jan, 2024)
- **Clinical breakpoints - fungi**
- **Dosages (v 14.0)** - file for printing and screen (1 Jan, 2024)

The major changes between the 2023 and 2024 breakpoint tables are:

- Fosfomycin iv breakpoints revised
- Cefiderocol ATUs revised, and zone diameter breakpoint for Enterobacterales adjus
- Ciprofloxacin breakpoints for staphylococci revised
- Breakpoint for C. difficile and fidaxomicin added
- Breakpoints for Bacillus anthracis added
- Breakpoints for Brucella melitensis added
- PK-PD breakpoints removed from the table (see explanation in the PK-PD tab) and "When there are no breakpoints"

European Committee on Antimicrobial Susceptibility Testing

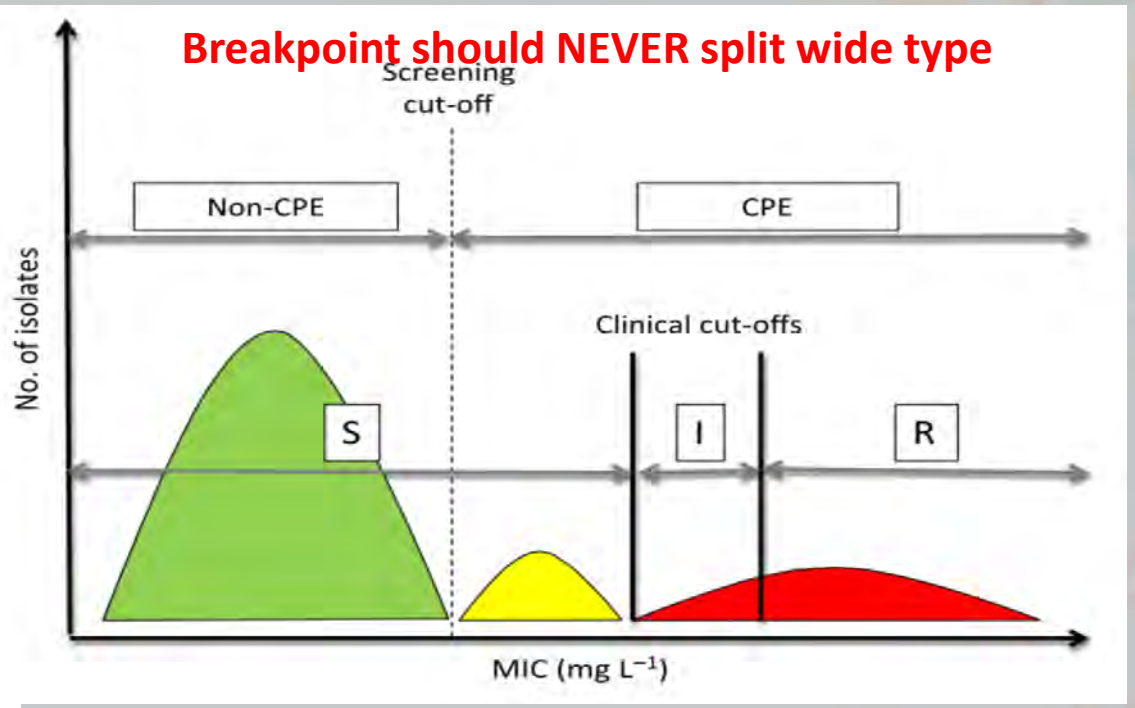
Breakpoint tables for interpretation of MICs and zone diameters

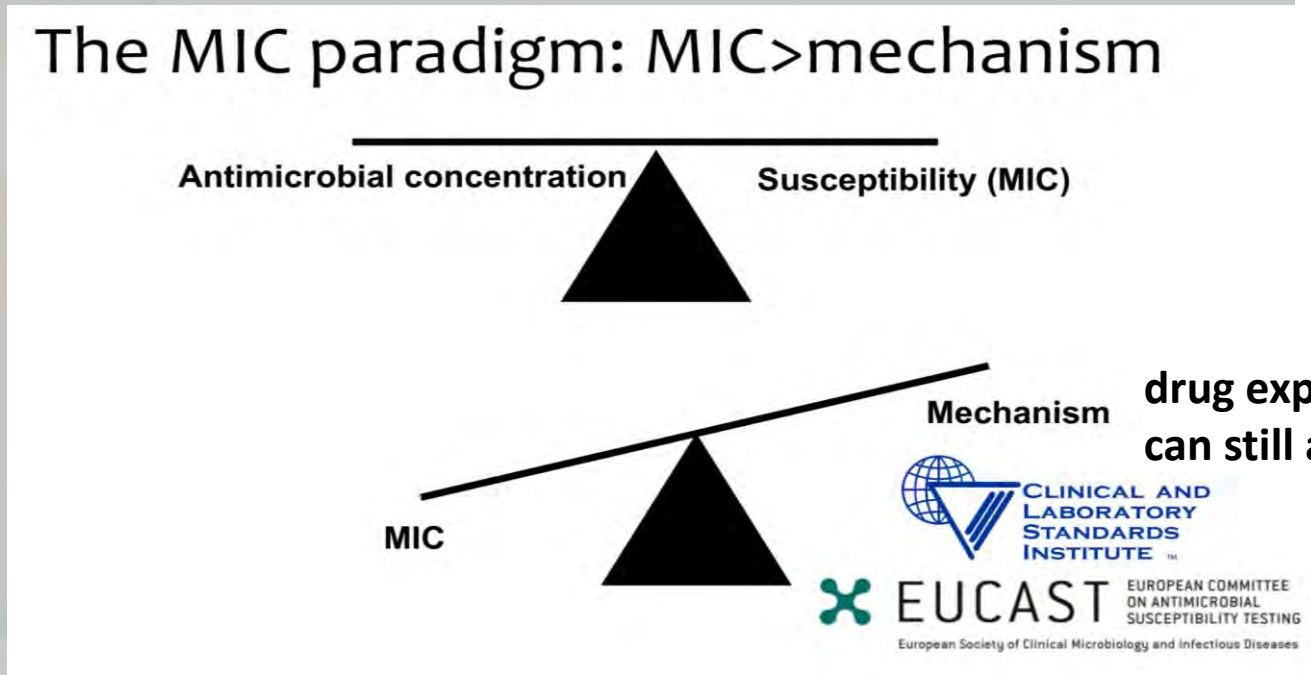
Version 14.0, valid from 2024-01-01

This document should be cited as "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. http://www.eucast.org."

EUCAST system-breakpoint

- **ECOFF** MIC distribution
- **PK/PD cut-off(s)**
- Clinical outcome data
  to confirm that indications and dosage regimens are correct



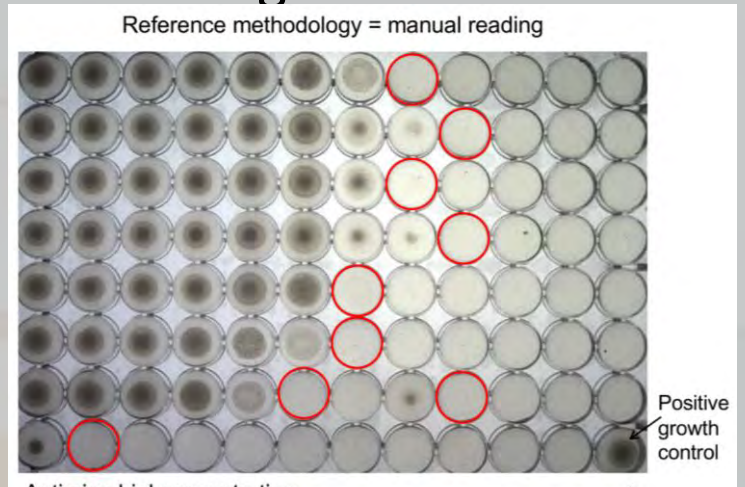


drug exposure higher without significant toxicity, can still achieve killing of low resistant organism

EUCAST Reference method- ISO 20776-2:2021

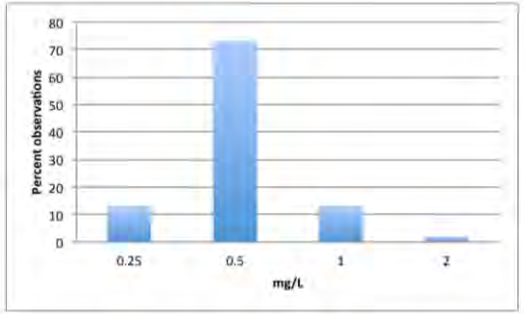
ISO 20776-2:2021

- **Broth microdilution technique**
  - Rapidly growing aerobic bacteria
  - 2.5-5% lysed horse blood for *Streptococcus* spp.
- agar dilution method
  - Anaerobes
  - Fastidious organisms such as *Neisseria* spp.

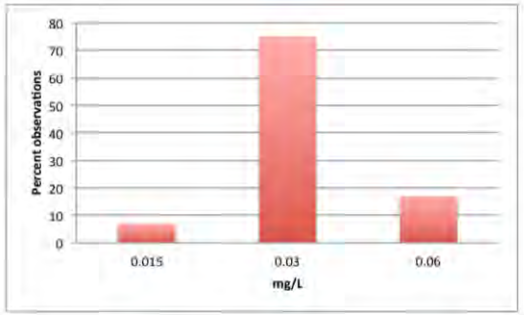


Reproducibility of broth microdilution

K. pneumoniae



E. coli



Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target[1]	Range[2]		Target[1]	Range[3]
Amikacin	1-2	0.5-4	30	22-23	19-26
Amoxicillin	4	***2-8***	-	-	-
Amoxicillin-clavulanic acid[4,5]	4	2-8	20-10	21	18-24[6]
Ampicillin	4	2-8	10	18-19	15-22[6]
Ampicillin-sulbactam[5,7]	***2***	***1-4***	10-10	21-22	19-24[6]
Aztreonam	0.125	0.06-0.25	30	32	28-36
Cefadroxil	-	-	30	***17***	***14-20***
Cefalexin	8	4-16	30	***18***	***15-21***
Cefepime	0.03-0.06	0.016-0.125	30	34	31-37
Cefixime	0.5	0.25-1	5	***23***	***20-26***
Cefotaxime	0.06	0.03-0.125	5	***28***	***25-31***
Cefoxitin	4	2-8	30	26	23-29
Cefpodoxime	0.5	0.25-1	10	25-26	23-28
Ceftaroline	0.06	0.03-0.125	5	***27***	***24-30***
Ceftazidime	0.125-0.25	0.06-0.5	10	***26***	***23-29***
Ceftazidime-avibactam[8,9]	0.125-0.25	0.06-0.5	10-4	***27***	***24-30***
Ceftibuten	0.25	0.125-0.5	30	31	27-35
Ceftobiprole	0.06	0.03-0.125	5	***28***	***25-31***
Ceftolozane-tazobactam[10,11]	0.25	0.125-0.5	30-10	28	24-32
Ceftriaxone	0.06	0.03-0.125	30	32	29-35
Cefuroxime	4	2-8	30	23	20-26
Chloramphenicol	4	2-8	30	24	21-27
Ciprofloxacin	0.008	0.004-0.016	5	***33***	***29-37***
Colistin[12]	0.5-1	0.25-2	-	-	-

Clinical consequences of very major errors with semi-automated testing systems for antimicrobial susceptibility of carbapenem-resistant Enterobacterales

Michele Bartoletti [1], Alberto Antonelli [2], Linda Bussini [3], Silvia Corcione [4], Daniele Roberto Giacobbe [5], Lorenzo Marconi [3], Renato Pascale [3], Silvia Dettori [5], Nour Shbaklo [6], Simone Ambretti [7], Paolo Gaibani [7], Tommaso Giani [2], Marco Coppi [2], Matteo Bassetti [5], Francesco Giuseppe De Rosa [8], Anna Marchese [9], Rossana Cavallo [10], Russell Lewis [11], Gian Maria Rossolini [2], Pierluigi Viale [12], Maddalena Giannella [12]

Objectives: In this study we investigated the rate of susceptibility testing discrepancies between semi-automated and reference systems with carbapenem-resistant Enterobacterales (CRE) and the impact of alleged errors by semi-automated systems on guiding targeted therapy for CRE bloodstream infection (BSI).

Methods: This was a multicentre, retrospective study enrolling patients with monomicrobial BSI caused by CRE from January 2013 to December 2016. Nonduplicate isolates from index blood cultures tested locally with semi-automated systems were centralized at a referral laboratory and retested with a reference broth microdilution or agar dilution method.

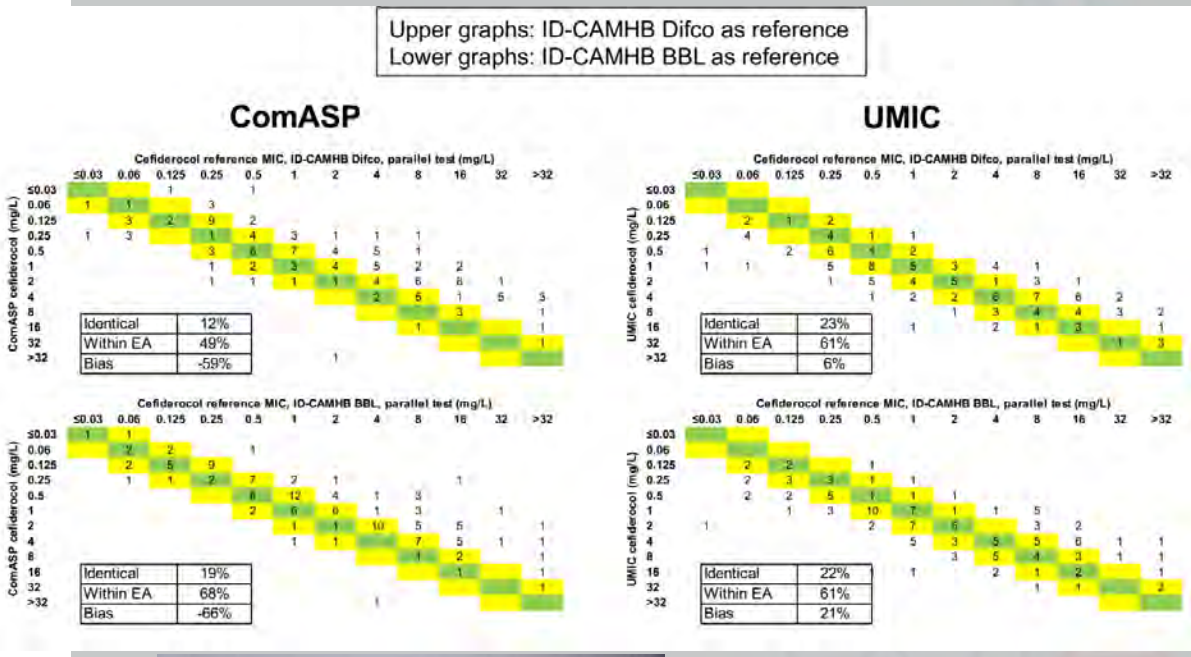
Results: We enrolled 366 patients with CRE-BSI; 220 (60%) were male, and the median age was 67 years (interquartile range, 54-76 years). When compared with the results of the reference methods, those of the semi-automated systems exhibited variable rates of very major errors (VMEs; i.e. false susceptibilities) and major errors (MEs; i.e. false resistances). The highest rates of VMEs were observed with fosfomycin (14%) and colistin (13.9%), and the highest rates of MEs were observed with gentamicin (21%), fosfomycin (7.7%), and tigecycline (34%). Overall, VMEs and MEs led clinicians to prescribe or confirm ineffective therapy in 25 of 341 patients (7%). Receipt of ineffective therapy supported by a misleading susceptibility test was associated with higher 30-day mortality rates by Kaplan-Meier survival curves rates compared with receipt of active therapy (56% vs. 26%; p = 0.002), and the difference was confirmed after adjustment for confounders in a Cox regression model (adjusted hazard ratio: 2.91; 95% CI, 1.62-5.22; p < 0.001).

Discussion: MEs and VMEs were relatively common with semi-automated susceptibility testing systems. VMEs were associated with inappropriate use of antibiotics and poorer outcomes.

Bartoletti M et al. *Clin Microbiol Infect*. 2022

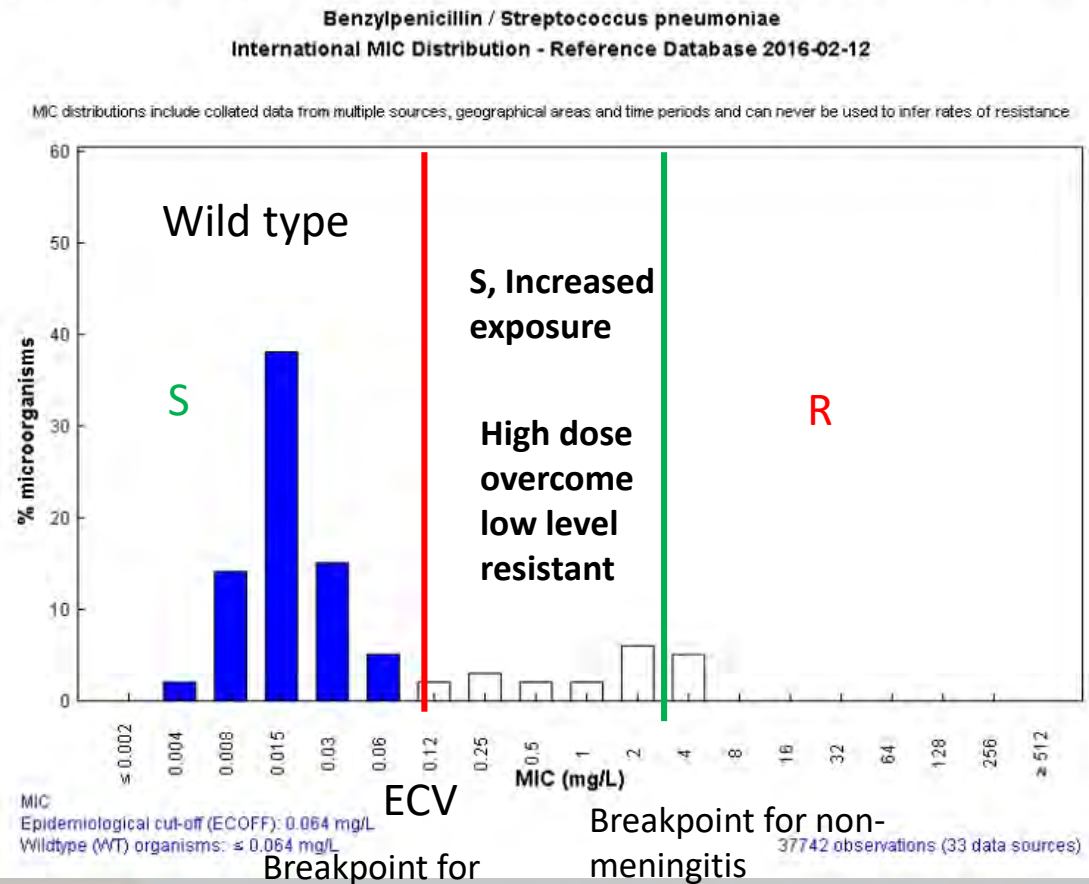
ISO 20776-2 (2021) - Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices -
Part 2: Evaluation of performance of antimicrobial susceptibility test devices against reference broth micro-dilution.

Upper graphs: ID-CAMHB Difco as reference
Lower graphs: ID-CAMHB BBL as reference



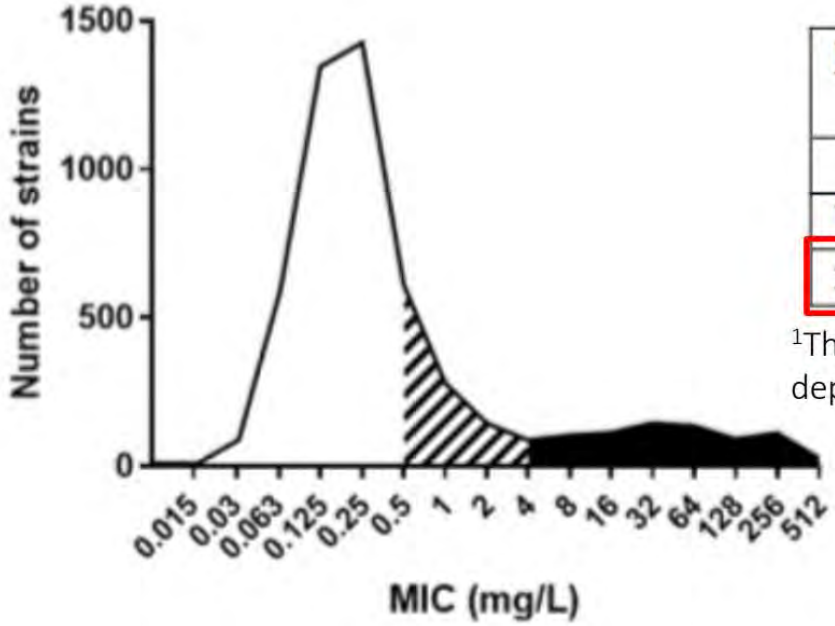
MIC variation among wild type

Figure 1: Benzylpenicillin MIC distributions for *Streptococcus pneumoniae*.



- range of MICs within the wild type is largely a consequence of technical variation
- biological differences playing lesser part.
- normal for the wild type MIC distribution to span 3-5 two-fold dilution steps
- Breakpoints should not split wild type MIC
- Reporting wild type MIC is NOT very useful →reflect technical variability
- Even less useful when unvalidated method other than ISO standard BMD is used

use of an MIC obtained by a single MIC determination is inappropriate especially within the ECOFF



MIC found	Interpretation for target attainment
Within WT, ≤ ECOFF	ECOFF
>ECOFF	MIC + two 2-fold dilutions [1]

[1]The number of dilutions could be higher or lower than two depending on proficiency of the lab and the drug-species distribution

- Variability: both technical and biological

- routine clinical laboratories cannot determine MICs with sufficient accuracy to guide dosage due to inherent assay variation in the MIC test

Mouton JW et al. J Antimicrob Chemother. 2018

1. How to interpret the EUCAST breakpoint table

CLSI

I – **Intermediate** … includes **isolates with MICs within the intermediate range** that approach usually attainable blood and tissue levels and/or for which response **rates may be lower than for susceptible isolates**

It also includes a **buffer zone** for inherent variability in test methods, which should prevent **small, uncontrolled, technical factors** from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

≠

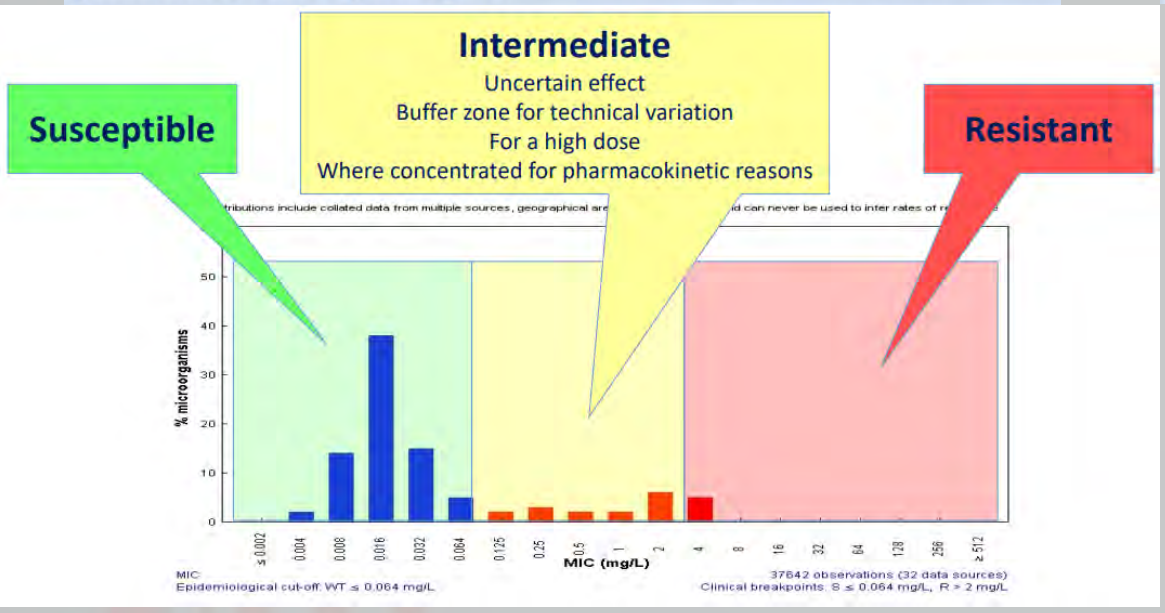
EUCAST

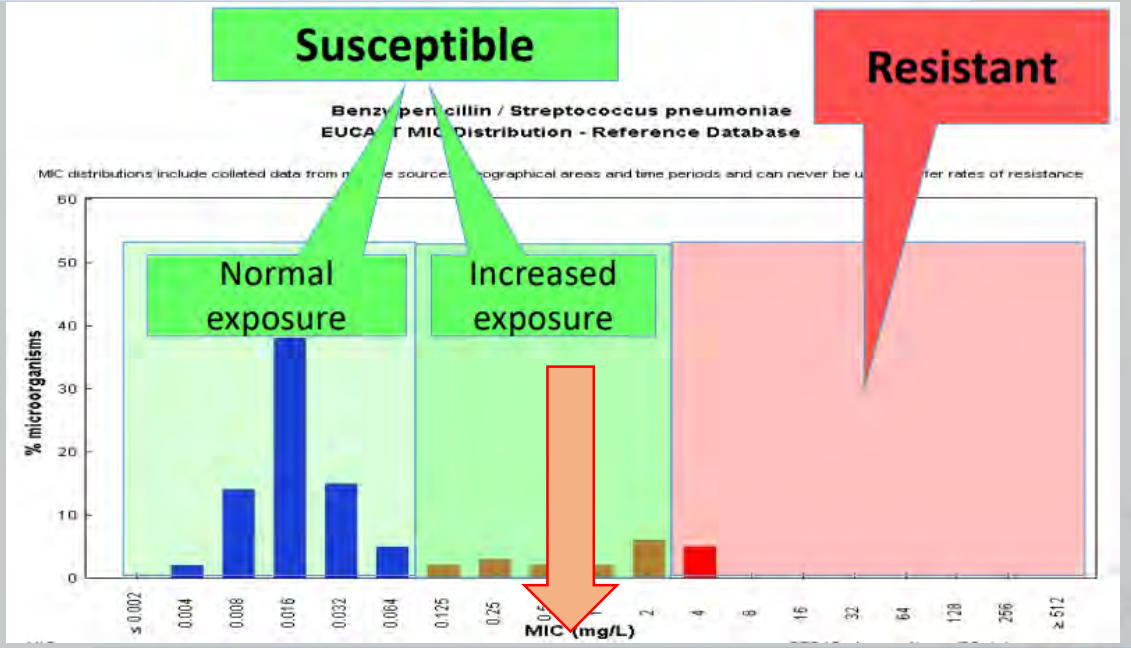
I – **Susceptible, increased exposure*** … when there is a **high likelihood of therapeutic success** because **exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.**

Improve PK/PD by at least one MIC step

SDD

*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.





This will affect your antibiogram!

- **Increased individual dose / higher frequency of dosing**
- **Mode of administration (oral to IV, injection to infusion)**
- **Physiological concentration of the agent at the site of infection (urine)**

Pseudomonas aeruginosa breakpoints v13.0

Arbitrary valued to assure that all susceptible population is categorized as "I"

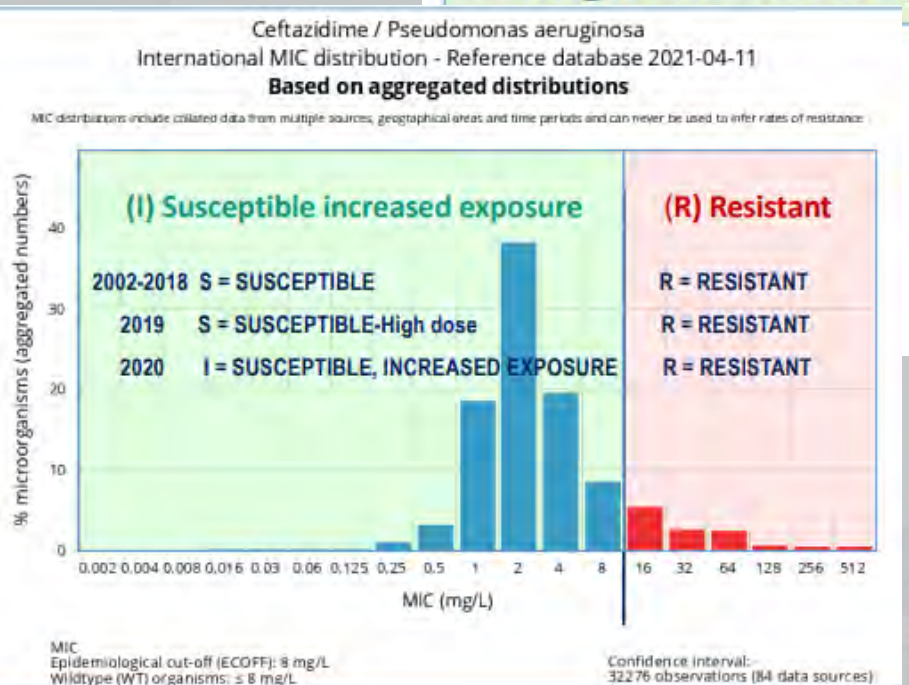
Beta-lactam agent	MIC breakpoints (mg/L)	
	S ≤	R >
Piperacillin	0.001	16
Piperacillin-tazobactam	0.001[1]	16[1]
Ticarcillin	0.001	16
Ticarcillin-clavulanic acid	0.001[2]	16[2]
Cefepime	0.001	8
Cefiderocol	2	2
Ceftazidime	0.001	8
Ceftazidime-avibactam	8[1]	8[1]
Ceftobiprole	IE	IE
Ceftolozane-tazobactam	4[1]	4[1]
Doripenem	0.001	2
Imipenem	0.001	4
Impenem-relebactam	2[1]	2[1]
Meropenem	2	8
Meropenem-vaborbactam	8[3]	8[3]

Breakpoints that categorise WT organisms as **"Susceptible, increased exposure" (I)** instead of "Susceptible, standard dosing regimen (S)" with HE superscript (high exposure)

Categorization as I / R

Those with **only one dose** are categorized as **S / R** (with no **I** category)

Those with **two doses, standard and high,** are categorized **S / I / R**



Cephalosporins	Standard dosage	High dosage
Cefepime	1 g x 3 iv or 2 g x 2 iv	2 g x 3 iv
Cefiderocol	2 g x 3 iv over 3 hours	None
Cefixime	0.2-0.4 g x 2 oral	None
Cefotaxime	1 g x 3 iv	2 g x 3 iv
Cefpodoxime	0.1-0.2 g x 2 oral	None
Ceftaroline	0.6 g x 2 iv over 1 hour	0.6 g x 3 iv over 2 hours
Ceftazidime	1 g x 3 iv	2 g x 3 iv or 1 g x 6 iv
Piperacillin-tazobactam	(4 g piperacillin + 0.5 g tazobactam) x 4 iv 30-minute infusion or x 3 iv by extended 4-hour infusion	(4 g piperacillin + 0.5 g tazobactam) x 4 iv by extended 3-hour infusion

Breakpoint interpretation

Disk contents in EUCAST and CLSI are mostly identical but exceptions occur

Fluoroquinolones	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		S ≥	R <	ATU
Ciprofloxacin	0.001	1		5	50	21	
Delafloxacin	IE	IE				IE	
Levofloxacin	0.5	1		5	23	20	
Moxifloxacin	-	-				-	
Nalidixic acid (screen only)	NA	NA					
Norfloxacin (uncomplicated UTI only)	-	-					
Ofloxacin	-	-			-	-	
Amikacin (systemic infections)	(16)[1]	(16)[1]		30	(15)[A]	(15)[A]	
Amikacin (infections originating from the urinary tract)	16	16		30	15	15	
Gentamicin (systemic infections)	IE	IE			IE	IE	
Gentamicin (infections originating from the urinary tract)	IE	IE			IE	IE	
Netilmicin	IE	IE			IE	IE	
Tobramycin (systemic infections)	(2)[1]	(2)[1]		10	(18)[A]	(18)[A]	
Tobramycin (infections originating from the urinary tract)	2	2		10	18	18	

S: standard dosage

Insufficient data, no breakpoint

NOT an agent for treatment of PAER

No standard dose regime suggested, use high dose regime

DD zone size 20-22: S, increased exposure

AGENT NOT suitable for treatment, AST not recommended; can considered as resistant if ST requested

Breakpoints in brackets distinguish between isolates without and with phenotypically detectable resistance based on ECOFFs

but for a specific indication **clinical evidence as monotherapy is usually lacking** or in combination with another active agent or measure they may still be used.

Isolates with resistance can be reported R

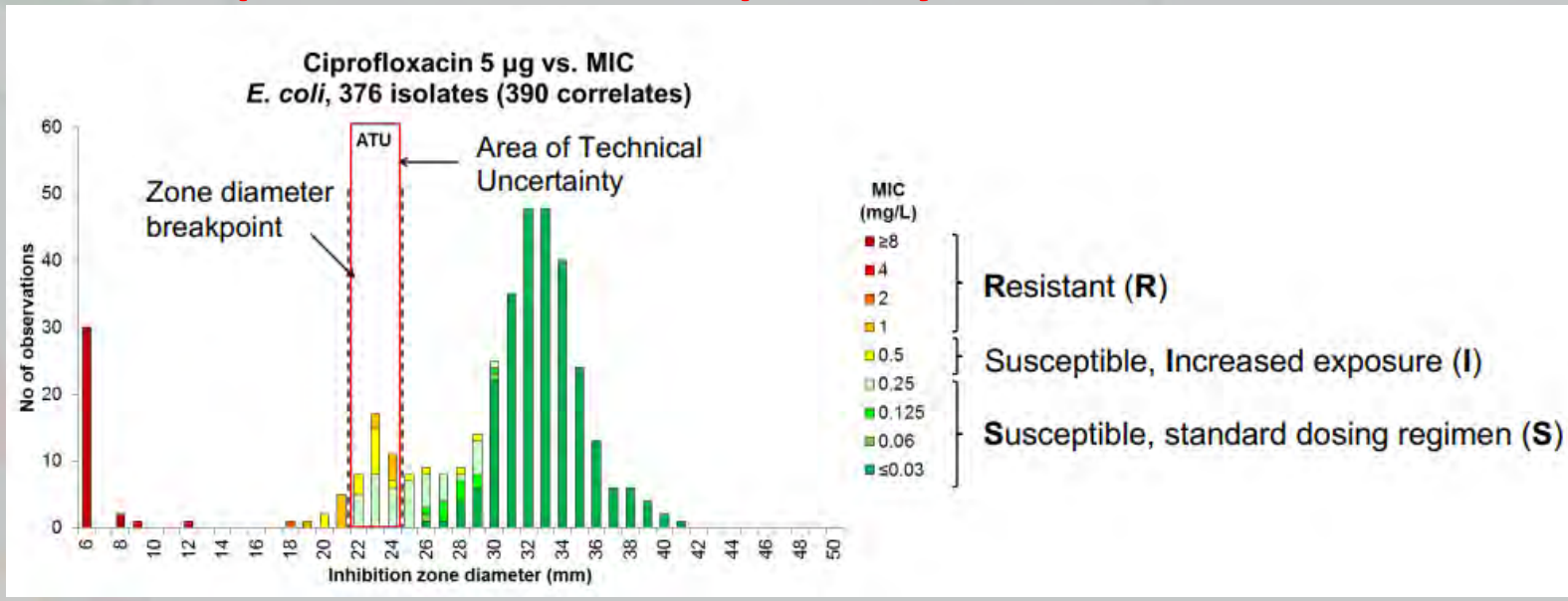
Reporting S or I if considered necessary, there should be a comment to explain the need for adjunctive measures.

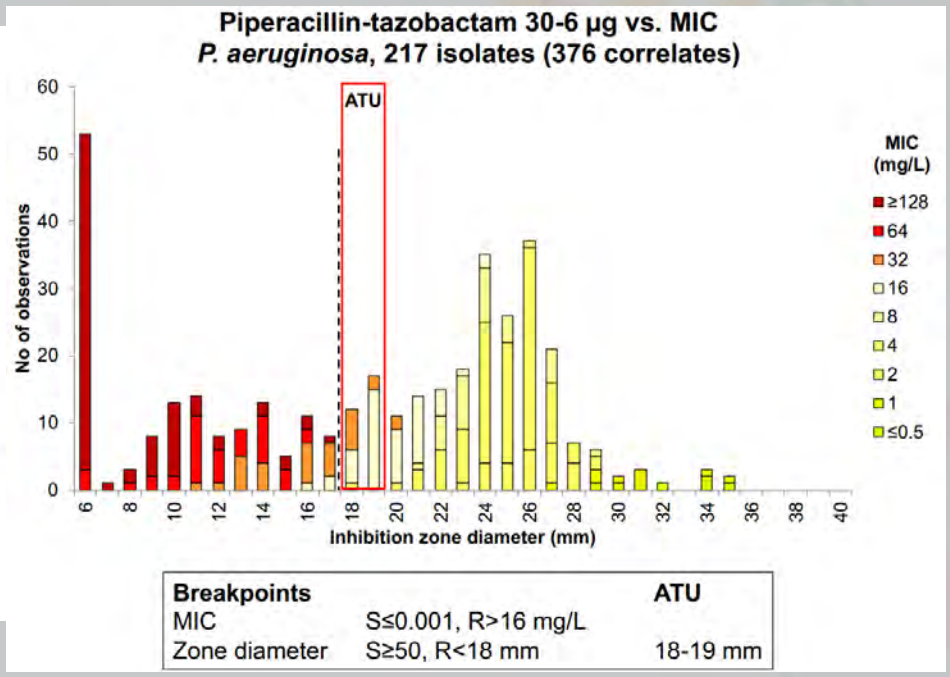
Recommended drug regime in EUCAST

Fluoroquinolones	Standard dosage	High dosage
Ciprofloxacin	0.5 g x 2 oral or 0.4 g x 2 iv	0.75 g x 2 oral or 0.4 g x 3 iv
Delafloxacin	0.45 g x 2 oral or 0.3 g x 2 iv	None
Levofloxacin	0.5 g x 1 oral or 0.5 g x 1 iv	0.5 g x 2 oral or 0.5 g x 2 iv
Moxifloxacin	0.4 g x 1 oral or 0.4 g x 1 iv	None
Norfloxacin	None	None
Ofloxacin	0.2 g x 2 oral or 0.2 g x 2 iv	0.4 g x 2 oral or 0.4 g x 2 iv

ATU: area of technical uncertainty

to prevent false susceptibility



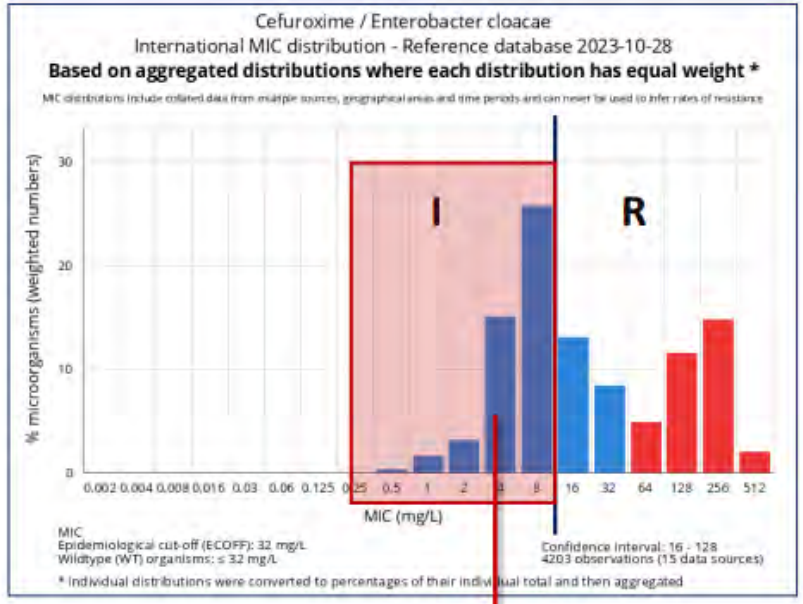


Breakpoints		ATU
MIC	S≤0.001, R>16 mg/L	
Zone diameter	S≥50, R<18 mm	18-19 mm

How to handle?

- Repeat the test – only if a technical error is suspected
- **Perform an alternative test – perform an MIC or a genotypic test**
- **Downgrade the susceptibility category – from S→I, I→R or S→R**
- Include the uncertainty as part of the report – categorise according to the breakpoints and add a comment on uncertainty
- Omit an uncertain result – report blank with a comment on uncertainty if alternative agent available

Expected resistant phenotypes



Rule	Organisms	Ampicillin/Amoxicillin	Amoxicilin-clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Cefazolin, Cephalothin Cefalexin, Cefadroxil	Cefoxitin[2]	Cefuroxime	Tetracyclines	Tigecycline	Polymyxin B, Colistin	Fosfomycin	Nitrofurantoin
1.1	*Citrobacter koseri, Citrobacter amalonaticus*[3]	R			R								
1.2	*Citrobacter freundii*[4]	R	R	R		R	R						
1.3	*Enterobacter cloacae* complex	R	R	R		R	R						
1.4	*Escherichia hermannii*	R			R								
1.5	*Hafnia alvei*	R	R	R		R	R				R		
1.6	*Klebsiella aerogenes*	R	R	R		R	R						
1.7	*Klebsiella oxytoca*	R			R								
1.8	*Klebsiella pneumoniae* complex[5]	R			R								
1.9	*Leclercia adecarboxylata*											R	
1.10	*Morganella morganii*	R	R	R		R			R		R		R
1.11	*Plesiomonas shigelloides*	R	R	R									
1.12	*Proteus mirabilis*								R	R	R		R
1.13	*Proteus penneri*	R				R		R	R	R	R		R

Expert rule IF susceptible to cefuroxime, THEN report cefuroxime and/or any other 2nd generation cephalosporin as resistant

Table 4 Expected resistant phenotype (susceptibility not expected) in gram-positive bacteria. Gram-positive bacteria are expected to be resistant to aztreonam, temocillin, polymyxin B/colistin and nalidixic acid.

Rule	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Macrolides	Clindamycin	Quinupristin-dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulfonamides
4.1	*Staphylococcus saprophyticus*	R	R								R	R	
4.2	*Staphylococcus cohnii*		R									R	
4.3	*Staphylococcus xylosus*		R									R	
4.4	*Staphylococcus capitis*		R								R		
4.5	Other coagulase-negative staphylococci and *S. aureus*		R										
4.6	*Streptococcus* spp.	R	R		R[1]								
4.7	*Enterococcus faecalis*	R	R	R	R[1]	R	R	R					R
4.8	*Enterococcus gallinarum, Enterococcus casseliflavus*	R	R	R	R[1]	R	R	R	R				R
4.9	*Enterococcus faecium*	R	R	R	R[1,2]	R							R
4.10	*Corynebacterium* spp.										R		
4.11	*Listeria monocytogenes*		R	R									
4.12	*Leuconostoc* spp., *Pediococcus* spp.								R	R			
4.13	*Lactobacillus* spp. (*L. casei, L. casei* var. *rhamnosus*)								R	R			

[1] Low-level resistance (LLR) to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against

Expected susceptible phenotypes

Table 1 Expected susceptible phenotype (resistance not expected) in gram-negative bacteria

Rule	Organisms	Unusual phenotypes
1.1	Any *Enterobacterales* (except *Morganellaceae* and *Serratia marcescens*)	Resistant to colistin[1,2]
1.2	*Salmonella* Typhi	Resistant to carbapenems
1.3	*Pseudomonas aeruginosa* and *Acinetobacter* spp.	Resistant to colistin[1]
1.4	*Haemophilus influenzae*	Resistant to any third-generation cephalosporin, carbapenems, fluoroquinolones[3]
1.5	*Moraxella catarrhalis*	Resistant to any third-generation cephalosporin or fluoroquinolones
1.6	*Neisseria meningitidis*	Resistant to any third generation cephalosporins or fluoroquinolones
1.7	*Neisseria gonorrhoeae*	Resistant to spectinomycin

Expert rules help problem solving in some ST dilemma

Fluoroquinolones							
8	Enterobacterales except *Salmonella* spp.	ciprofloxacin	all fluoroquinolones	IF resistant to ciprofloxacin, THEN report as resistant to all fluoroquinolones IF susceptible to ciprofloxacin, THEN report other fluoroquinolones as tested	Acquisition of at least two target mutations in either *gyr*A or *gyr*A plus *parC*. The AAC(6')-Ib-cr enzyme partially inactivates ciprofloxacin but not levofloxacin; however, with current breakpoints this difference cannot be detected	B	Cavaco et al., 2008; Martínez-Martínez, Eliecer Cano, Manuel Rodríguez-Martínez, Calvo, & Pascual, 2008

Rule No.	Organism(s)	Indicator Agent	Agent(s) Affected*	Rule	Remarks	Grade	References
	Beta-lactams						
1	*Enterococcus faecalis and E. faecium*	Ampicillin	amoxicillin, ureidopenicillins and imipenem	IF resistant to ampicillin THEN report as resistant to ureidopenicillins and imipenem	Alterations in PBP5 lead to reduced affinity for beta-lactams. Although ampicilin resistance predicts the test result for imipenem, this is not true for ampicillin susceptibility. In *E. faecalis*, susceptibility to ampicillin, amoxicillin and piperacillin (with and without beta-lactamase inhibitor) can be inferred from ampicillin in ≥98% of isolates. In other *Enterococcus* spp. (including *E. faecium*), susceptibility to these agents is uncommon and isolates resistant to ampicillin should not be reported susceptible to either amoxicillin or piperacillin (with or without inhibitor)	C	

Carbapenems	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		S ≥	R <	ATU
Doripenem	-	-			-	-	
Ertapenem	-	-			-	-	
Imipenem	0.001	4		10	50	21	

2. How can EUCAST methods facilitate our ST testing?

Disk diffusion breakpoint available in some organisms only in EUCAST

B. pseudomallei

Penicillins	Disk content (µg)	Zone diameter breakpoints (mm)		
		S ≥	R <	ATU
Amoxicillin-clavulanic acid	20-10	50	22	
Cephalosporins	**Disk content (µg)**	**Zone diameter breakpoints (mm)**		
		S ≥	R <	ATU
Ceftazidime	10	50	18	
Carbapenems	**Disk content (µg)**	**Zone diameter breakpoints (mm)**		
		S ≥	R <	ATU
Imipenem	10	29	29	
Meropenem	10	24	24	

Bacillus spp. other than *B. anthracis*

Carbapenems	Disk content (µg)	Zone diameter breakpoints (mm)		
		S ≥	R <	ATU
Imipenem	10	30	30	
Meropenem	10	25	25	
Fluoroquinolones	**Disk content (µg)**	**Zone diameter breakpoints (mm)**		
		S ≥	R <	ATU
Ciprofloxacin	5	50[A]	23[A]	
Levofloxacin	5	50[A]	23[A]	
Norfloxacin (screen only)	10	21[B]	21[B]	
Glycopeptides	**Disk content (µg)**	**Zone diameter breakpoints (mm)**		
		S ≥	R <	ATU
Vancomycin	5	10[A]	10[A]	

Corynebacterium spp.

Penicillins	Disk content (µg)	Zone diameter breakpoints (mm)		
		S ≥	R <	ATU
Benzylpenicillin	1 unit	50	12	
Fluoroquinolones	**Disk content (µg)**	**Zone diameter breakpoints (mm)**		
		S ≥	R <	ATU
Ciprofloxacin	5	50	25	
Moxifloxacin	5	25	25	
Aminoglycosides	**Disk content (µg)**	**Zone diameter breakpoints (mm)**		
		S ≥	R <	ATU
Gentamicin		IE	IE	
Glycopeptides	**Disk content (µg)**	**Zone diameter breakpoints (mm)**		
		S ≥	R <	ATU
Vancomycin	5	17[A]	17[A]	
Macrolides and lincosamides	**Disk content (µg)**	**Zone diameter breakpoints (mm)**		
		S ≥	R <	ATU
Clindamycin[1]	2	20	20	

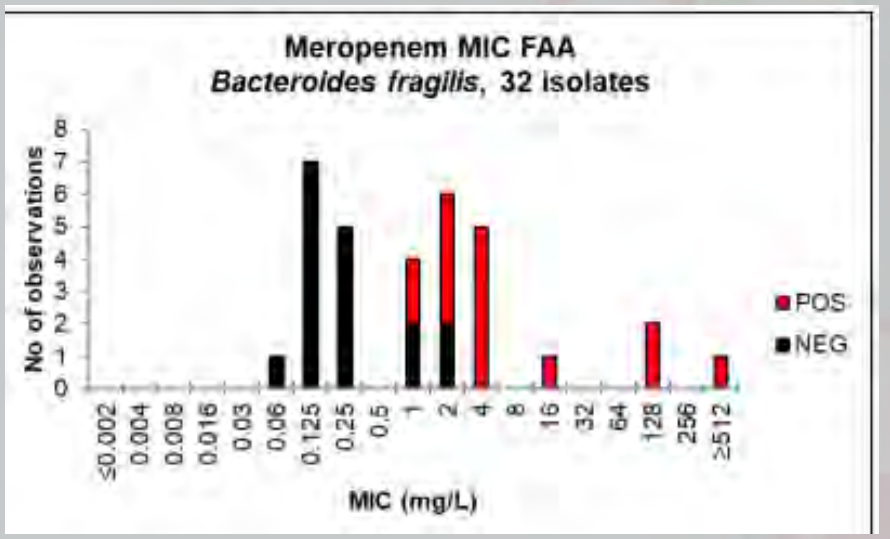
Aerococcus urinae

Penicillins	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		S ≥	R <	ATU
Benzylpenicillin	0.125	0.125		1 unit	21	21	
Ampicillin	0.25	0.25		2	26	26	
Amoxicillin	Note[1]	Note[1]			Note[A]	Note[A]	
Carbapenems	**MIC breakpoints (mg/L)**			**Disk content (µg)**	**Zone diameter breakpoints (mm)**		
	S ≤	R >	ATU		S ≥	R <	ATU
Meropenem	0.25	0.25		10	31	31	

Anaerobic ST

- Agar dilution as gold standard for both CLSI/EUCAST
- BMD only for *Bacteroides* in CLSI
- Increasing resistance esp for *Bacteroides* spp.
- Polymicrobial infections are common; consider whether all isolated anaerobes need testing

Metallo-beta-lactamase cfiA
nim-genes encoding nitroimidazole reductase



Testing Conditions

Medium: Agar dilution (for all anaerobes): Brucella agar supplemented with hemin (5 µg/mL), vitamin K_1 (1 µg/mL), and laked sheep blood (5% v/v)
Broth microdilution (for *Bacteroides* spp. and *Parabacteroides* spp. only): Brucella broth supplemented with hemin (5 µg/mL), vitamin K_1 (1 µg/mL), and LHB (5% v/v)

Inoculum: Broth culture method or colony suspension, equivalent to 0.5 McFarland suspension
Agar: 10^5 CFU per spot
Broth: 10^6 CFU/mL

Incubation: 36°C ± 1°C, anaerobically
Broth microdilution: 46-48 hours
Agar dilution: 42-48 hours

MIC determination (agar dilution)
Medium: Fastidious Anaerobe Agar + 5% defibrinated horse blood (FAA-HB)
Inoculum: 10^5 CFU/spot
Incubation: Anaerobic environment, 35-37°C, 48h
Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent where a noticeable difference is seen in visible growth between the test and control plate.
Quality control: *Bacteroides fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124.
For control of the inhibitor component of beta-lactam inhibitor combinations, see EUCAST QC Tables.
See disk diffusion methodology for how to monitor the anaerobic atmosphere with *Clostridium perfringens* DSM 25589.

CLSI: breakpoint same for different anaerobic spp.

Table 2J. Anaerobes (Continued)

Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL S	I	R
PENICILLINS			
Ampicillin	≤0.5	1	≥2
Penicillin	≤0.5	1	≥2
B-LACTAM COMBINATION AGENTS			
(10) Organisms that test susceptible to the B-lactam agent alone are also c susceptible to the B-lactam combination agent cannot be assumed to be su the B-lactam agent alone may be susceptible to the B-lactam combination			
Amoxicillin-clavulanate	≤4/2	8/4	≥16/8
Ampicillin-sulbactam	≤8/4	16/8	≥32/16
Piperacillin-tazobactam	≤16/4	32/4-64/4	≥128/4
Imipenem-relebactam	≤4/4	8/4	≥16/4
Ticarcillin-clavulanate*	≤32/2	64/2	≥128/2
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please			
Cefotetan	≤16	32	≥64
Cefoxitin	≤16	32	≥64
Ceftizoxime*	≤32	64	≥128
Ceftriaxone	≤16	32	≥64
Cefmetazole*	≤16	32	≥64
Cefoperazone*	≤16	32	≥64
Cefotaxime*	≤16	32	≥64

EUCAST: **Genus specific breakpoint**

Bacteroides spp.

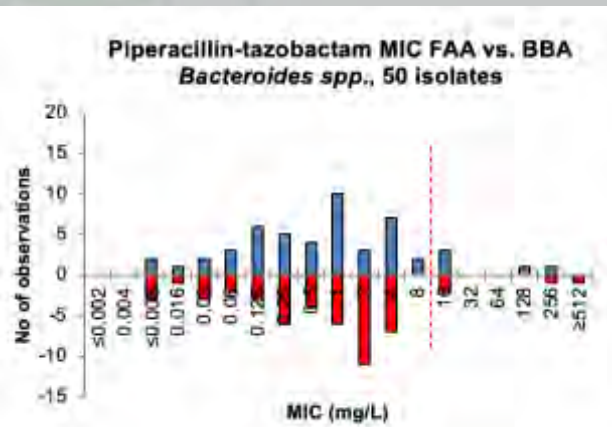
Breakpoints for *Bacteroides* spp. are also valid for *Parabacteroides* s

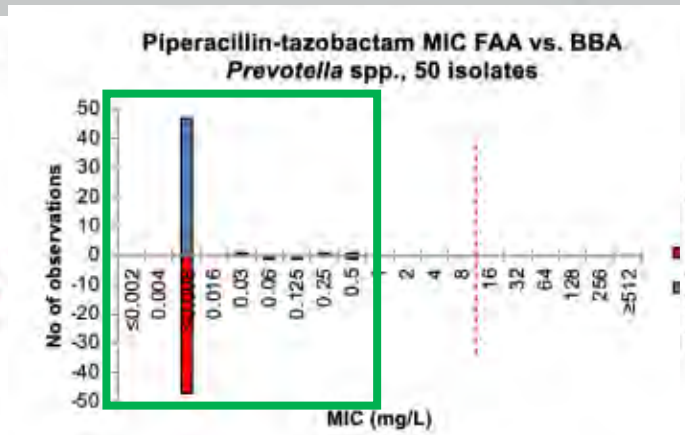
Antimicrobial agent	MIC breakpoints (mg/L) S ≤	R >	ATU
Ampicillin-sulbactam	2[1]	2[1]	
Amoxicillin-clavulanic acid	2[2]	2[2]	
Piperacillin-tazobactam	2[3]	2[3]	
Ertapenem	(2)[4]	(2)[4]	
Imipenem	1	1	
Meropenem	1	1	
Clindamycin	(4)[4]	(4)[4]	
Metronidazole	4	4	

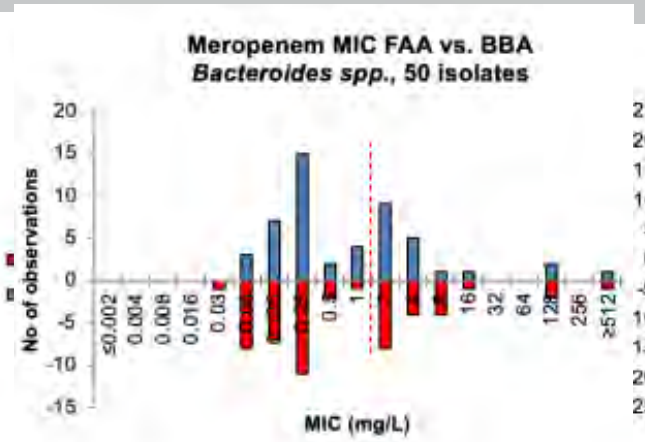
Prevotella spp.

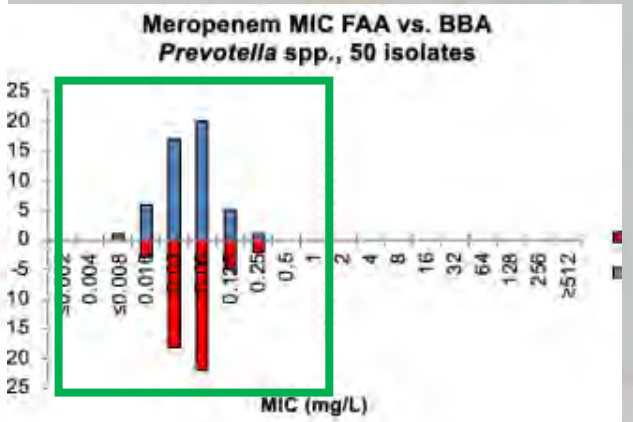
Antimicrobial agent	MIC breakpoints (mg/L) S ≤	R >	ATU
Benzylpenicillin	0.5[1]	0.5[1]	
Ampicillin	0.5[1]	0.5[1]	
Ampicillin-sulbactam	Note[1,2]	Note[1,2]	
Amoxicillin	0.25[1]	0.25[1]	
Amoxicillin-clavulanic acid	Note[1,2]	Note[1,2]	
Piperacillin-tazobactam	Note[1,2]	Note[1,2]	
Ertapenem	0.5[1]	0.5[1]	
Imipenem	0.125[1]	0.125[1]	
Meropenem	0.25[1]	0.25[1]	
Clindamycin	0.25	0.25	
Metronidazole	4	4	

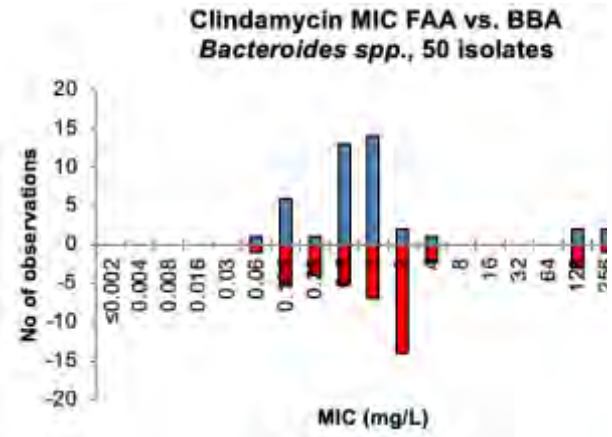
ECOFF of *Bacteroides* spp. are different from *Prevotella* spp.

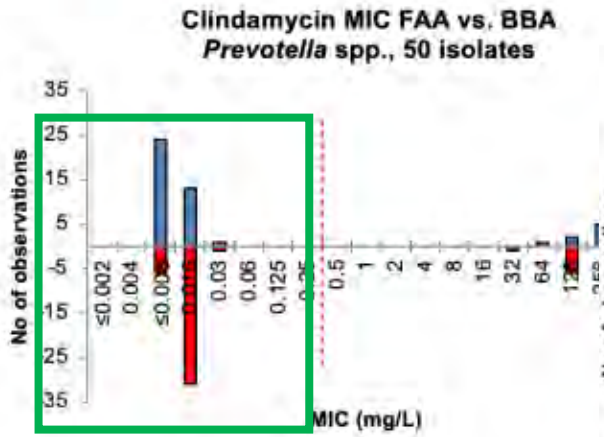


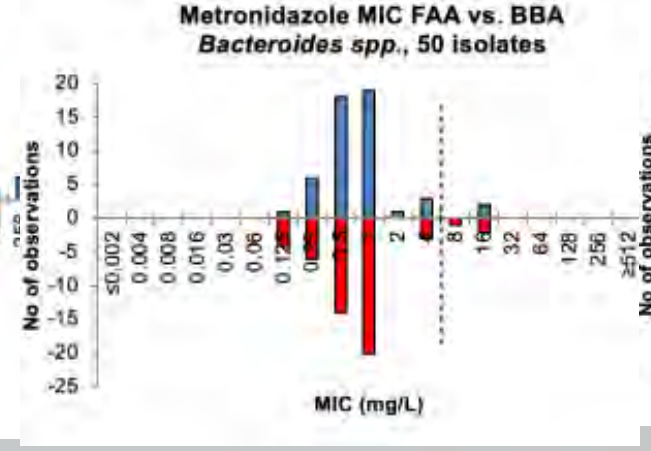


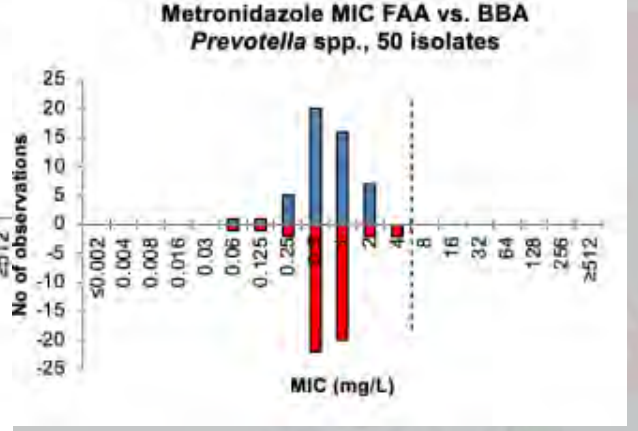






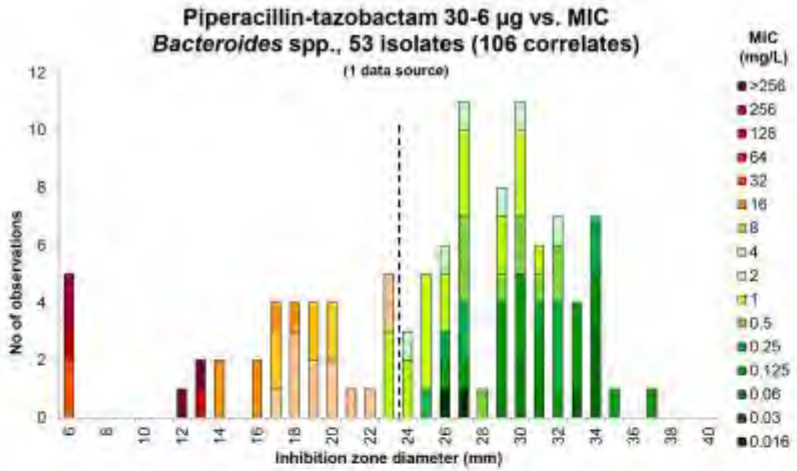




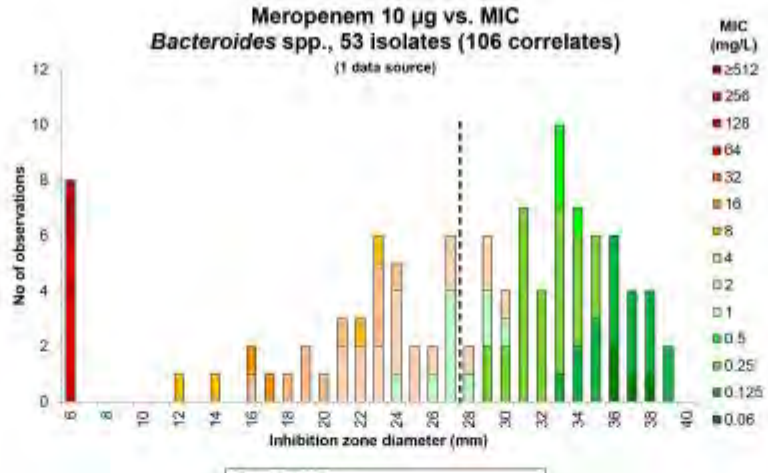


MIC distribution/ECOFF of organism is an important component of setting up breakpoint

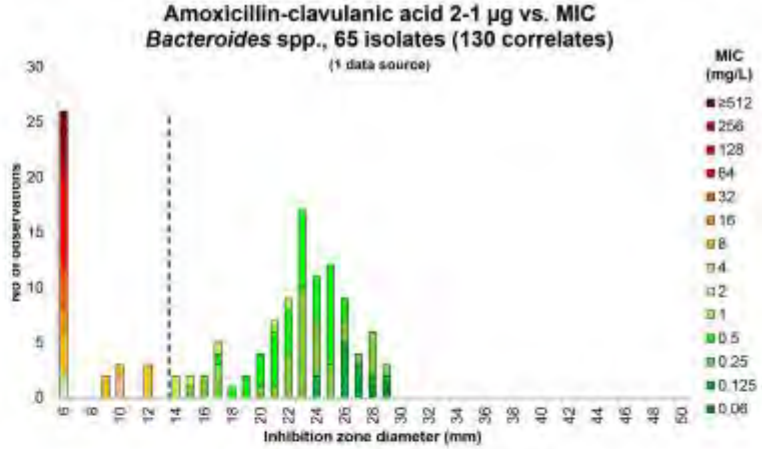
EUCAST: Examples of **calibration** vs **agar dilution**



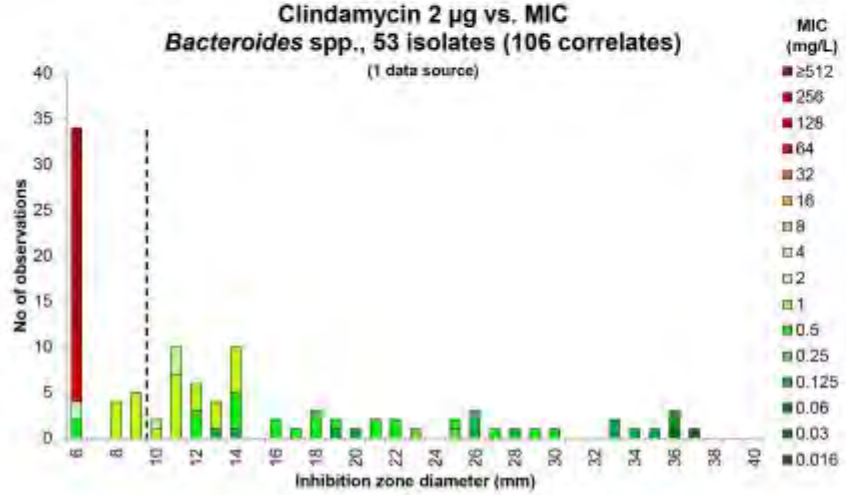
Breakpoints	
MIC	S≤2, R>2 mg/L
Zone diameter	S≥24, R<24 mm



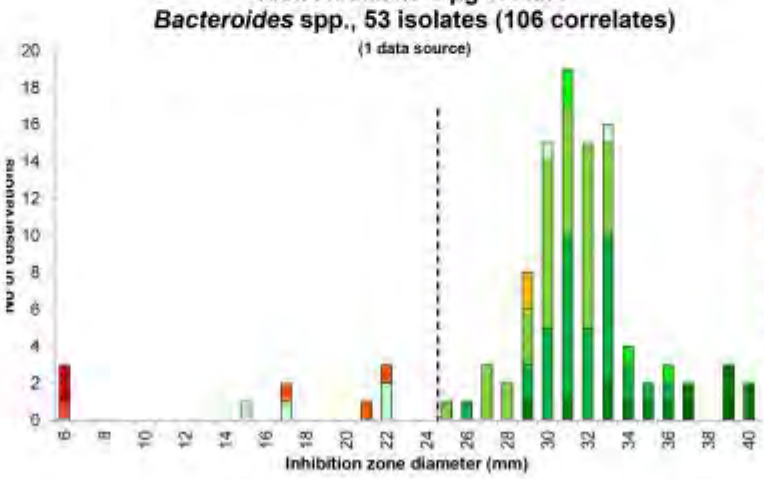
Breakpoints	
MIC	S≤1, R>1 mg/L
Zone diameter	S≥28, R<28 mm



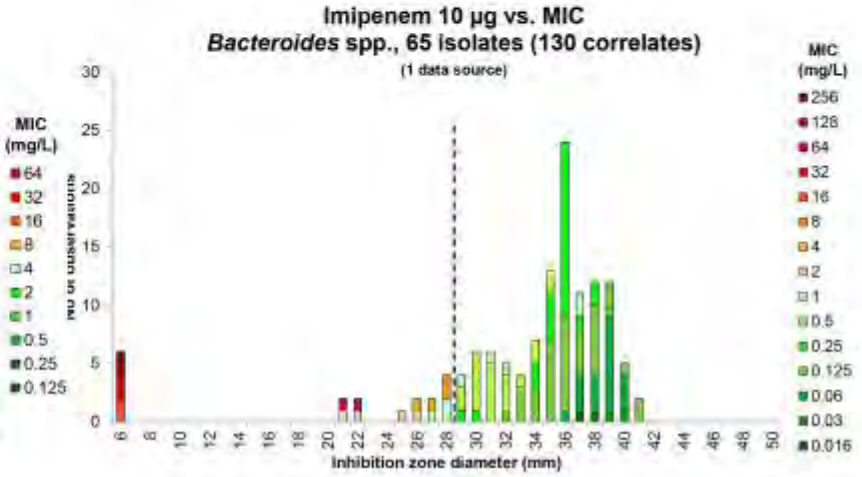
Breakpoints	
MIC	S≤2, R>2 mg/L
Zone diameter	S≥14, R<14 mm



Breakpoints	
MIC	S≤4, R>4 mg/L
Zone diameter	S≥10, R<10 mm



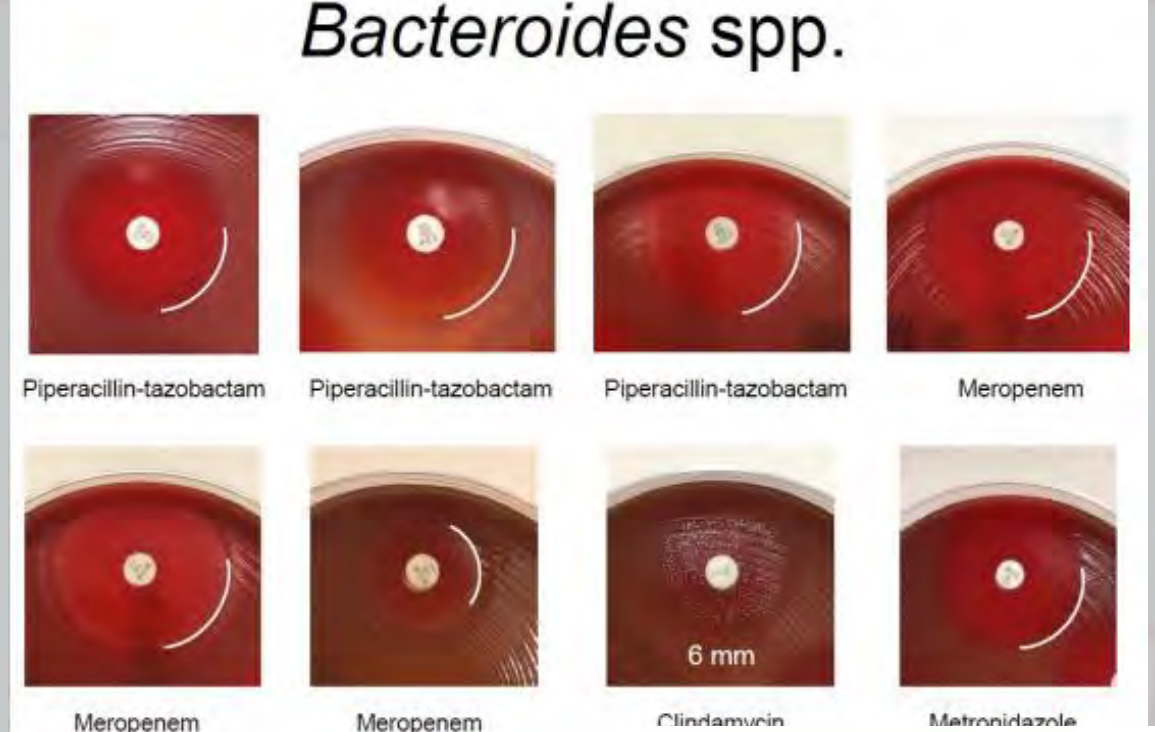
Breakpoints	
MIC	S≤4, R>4 mg/L
Zone diameter	S≥25, R<25 mm

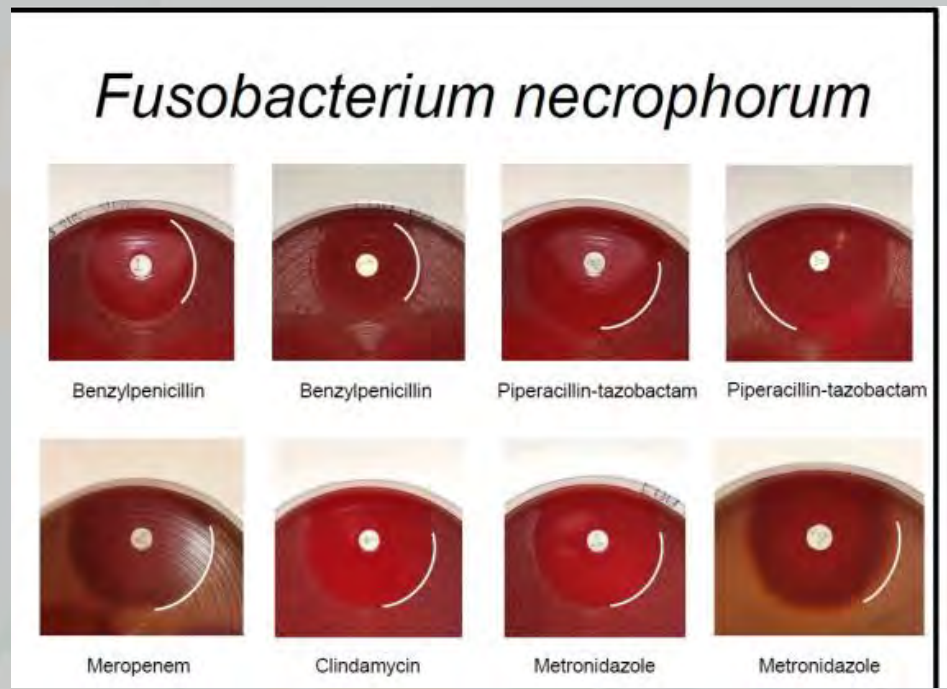


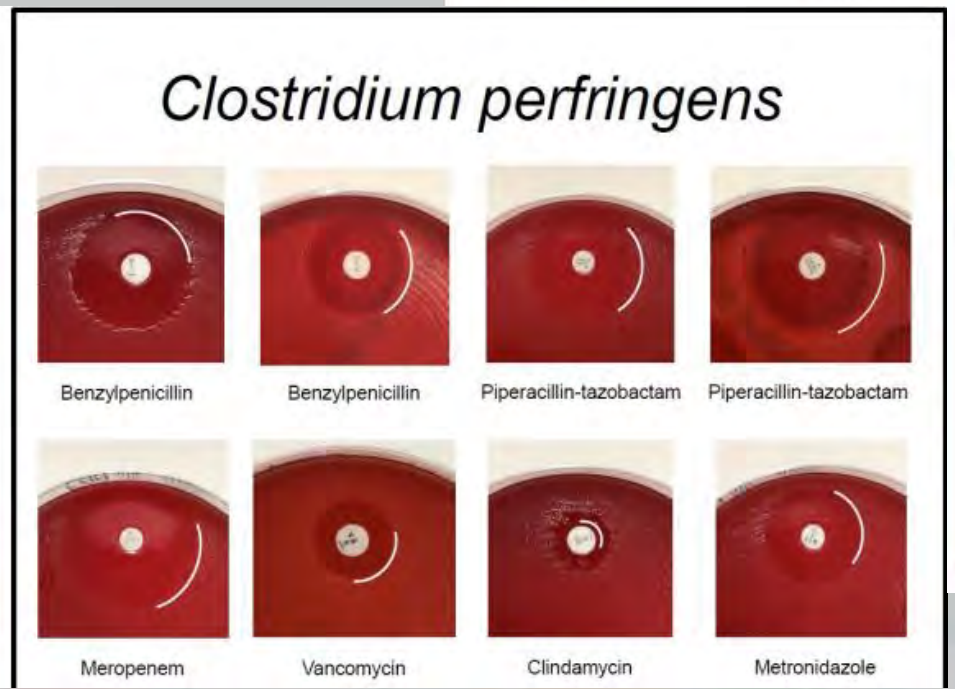
Breakpoints	
MIC	S≤1, R>1 mg/L
Zone diameter	S≥29, R<29 mm

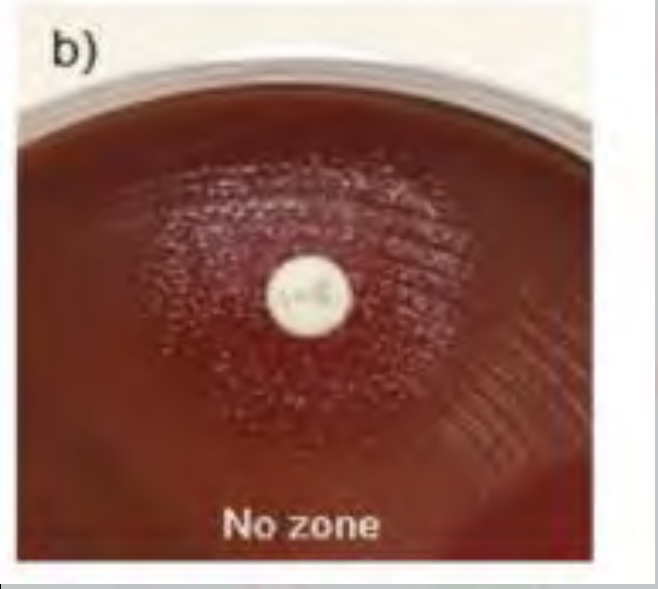
Anaerobic ST-technical requirement

- <u>Ignore any faint haze within the inhibition zone</u>
- read the most obvious zone.
- Ignore haemolysis
- Isolated colonies within the inhibition zone should be taken into account (esp clindamycin)
- ***<u>DO not extend incubation time</u>***









Disk diffusion (EUCAST standardised disk diffusion method)

Medium: Fastidious Anaerobe Agar + 5% defibrinated horse blood (FAA-HB). The plates should be dried prior to inoculation (at 20-25°C overnight or at 35°C, with the lid removed, for 15 min).

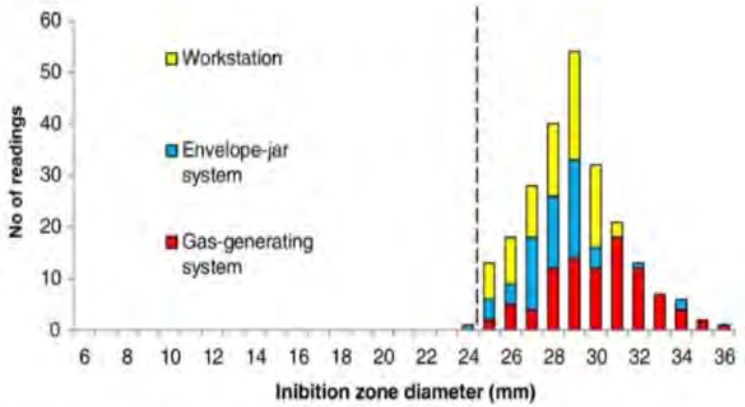
Inoculum: McFarland 1.0

Incubation: Anaerobic environment, 35-37°C, 18±2h

Reading: Unless otherwise stated, read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light. See pictures below and the EUCAST Reading Guide for disk diffusion of anaerobic bacteria for further information.

Quality control: *Bacteroides fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124. For control of the inhibitor component of beta-lactam inhibitor combination disks, see EUCAST QC Tables.

Clostridium perfringens DSM 25589 with a metronidazole 5 µg disk to monitor the anaerobic atmosphere.

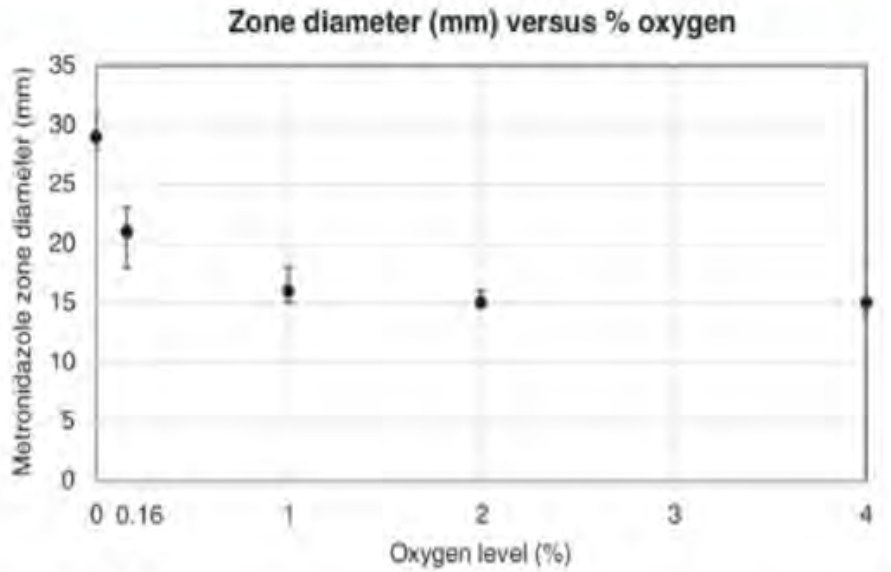


Reproducibility results from 18 laboratories and different anaerobic environments. Metronidazole 5 µg inhibition zone diameters on FAA-HB and *Clostridium perfringens* DSM 25589 (*n*=236)

Bacteroides spp.

Breakpoints for *Bacteroides* spp. are also valid for *Parabacteroides* spp. and for *Phocaeicola dorei/vulgatus* (

Antimicrobial agent	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		S ≥	R <	ATU
Ampicillin-sulbactam	2[1]	2[1]		10-10	25	25	
Amoxicillin-clavulanic acid	2[2]	2[2]		2-1	14	14	
Piperacillin-tazobactam	2[3]	2[3]		30-6	24	24	
Ertapenem	(2)[4]	(2)[4]		10	(23)[A]	(23)[A]	
Imipenem	1	1		10	29	29	
Meropenem	1	1		10	28	28	
Clindamycin	(4)[4]	(4)[4]		2	(10)[A,B]	(10)[A,B]	
Metronidazole	4	4		5	25	25	



Results from the first part of the study. The metronidazole 5 µg zone diameter (mm) on FAA-HB versus the oxygen level (%) in the Anoxomat jar system. The medians with ranges are shown (*n*=12 at each oxygen level)

Increased QC requirement

Control of the inhibitor component of β-lactam-inhibitor combinations

Test according to EUCAST methodology for non-fastidious organisms (MH broth and agar). See EUCAST Breakpoint Tables for short descriptions of MIC and disk diffusion methodology.

***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 (µg)	Inhibition zone diameter (mm)	
	Target[1]	Range[2]		Target[1]	Range[2]
Amoxicillin-clavulanic acid[3]	***8-16***	***4-32***	20-10	19-20	17-22[4]
Ampicillin-sulbactam[5]	***32-64***	***16-128***	10-10	16	13-19[4]
Ceftolozane-tazobactam[6,7]	0.125	0.06-0.25	30-10	★ 28	25-31
Piperacillin-tazobactam[6,7]	1	0.5-2	30-6	★ ***24***	***21-27***
Ticarcillin-clavulanic acid[3]	16	8-32	75-10	23	21-25

Klebsiella pneumoniae* ATCC 700603

(NCTC 13368, CCUG 45421, CECT 7787)

SHV-18 ESBL producer

* Two colony types are normally observed for this strain and should be included when subculturing and testing the strain.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target[1]	Range[2]		Target[1]	Range[2]
Ceftazidime-avibactam[8]	0.5-1	0.25-2	10-4	***21***	***18-24***
Ceftolozane-tazobactam[6,7]	1	0.5-2	30-10	★ 21	17-25
Piperacillin-tazobactam[6,7]	16	8-32	30-6	★ ***17***	***14-20***

- Ranges: allows for day to day testing variation
- Target :mean values from repeated measurements should be optimally ±1mm
- EUCAST recommends daily QC or at least 4 times/week

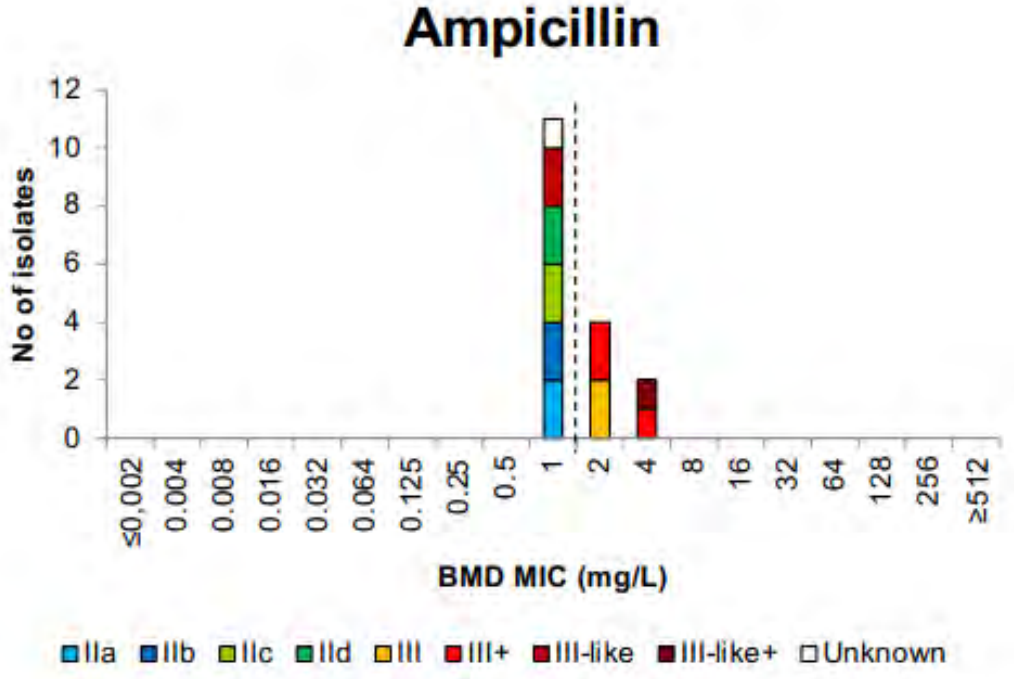
H. Influenzae

- **CLSI: removal of AUG disk diffusion breakpoint**
- Ampicillin/Augmentin ST discrepancy with cefuroxime

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	I	R	Interpretive Categories and MIC Breakpoints, µg/mL S	I	R	Comments
β-LACTAM COMBINATION AGENTS								
(13) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Ampicillin-sulbactam	10/10 µg	≥20		≤19	≤2/1		≥4/2	See comment (12). **(14)** Breakpoints are based on a dosage regimen of 3 g IV administered every 6 h.
Amoxicillin-clavulanate	20/10 µg				≤2/1	4/2	≥8/4	**(15)** Breakpoints are based on a dosage regimen of 875/125 mg orally administered every 12 h or 500/125 mg every 8 h. Additional disk correlate data are pending before disk diffusion breakpoints with this dosage regimen can be established. See general comment (6) and comment (12).

Detect Beta-lactamase negative HINF due to PBP3 mutation



Blue/green bars = PBP3 mutations group II

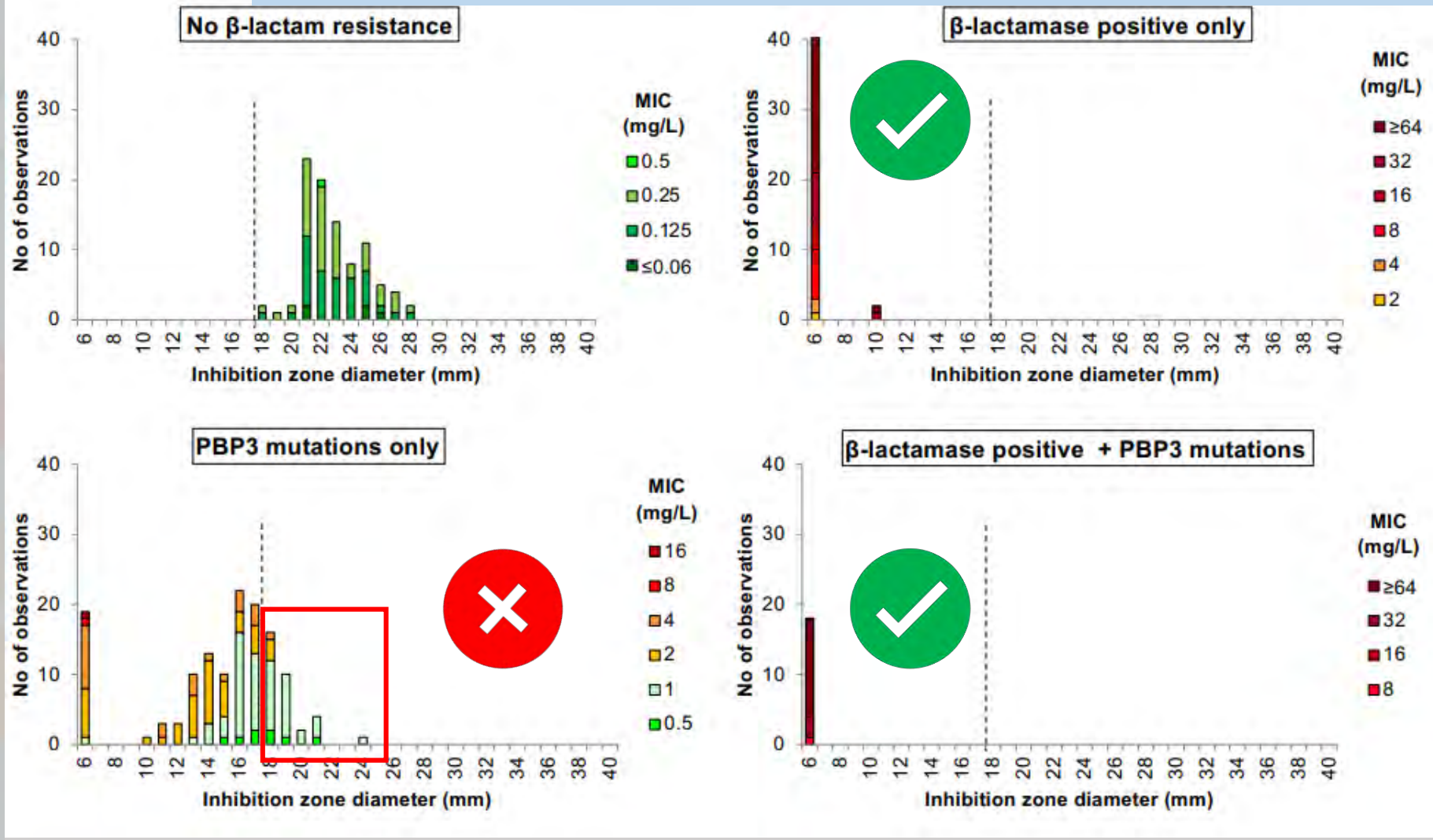
Main rPBP3 group	Subgroup according to Skaare [7]	Subgroup according to Ubukata [3] and Dabernat [6]	MIC range of Ampicillin (mg/L)
Group I			0.5–2 [5]
Group II			0.5–8 [5]
	A	IIb	
	B	IId	
	C	IIb	
	D	II-	
	E	IIc	
	F	IIa	
	G	II-	
Group III			1–32 [4]
Group III-like			0.5–2 [5]

Resistance due to changes in PBP3 can be defined as low-level

Strain ID	Geno-type	Zone diameter PCG 1 U (mm)	Screening phenotype[a]	Susceptible to amino-penicillins[b]	MIC[c] amoxicillin	MIC ampicillin	MIC amoxicillin clavulanic acid	MIC cefotaxime	MIC ceftriaxone	MIC cefuroxime	MIC imipenem	MIC mero-penem	β-lactamase
NTHi3655	Wild-type	16	Susceptible	Susceptible	0.5	≤0.25	≤0.25	≤0.015	≤0.015	1	0.5	0.06	Negative
NTHi3655-PBP3^{Y528H}	Y528H	11	Resistant	Susceptible	1	0.5	1	≤0.015	≤0.015	1	1	0.06	Negative
NTHi93–57485	Y528H	6	Resistant	Susceptible	1	1	1	0.06	≤0.015	4	0.5	0.06	Negative

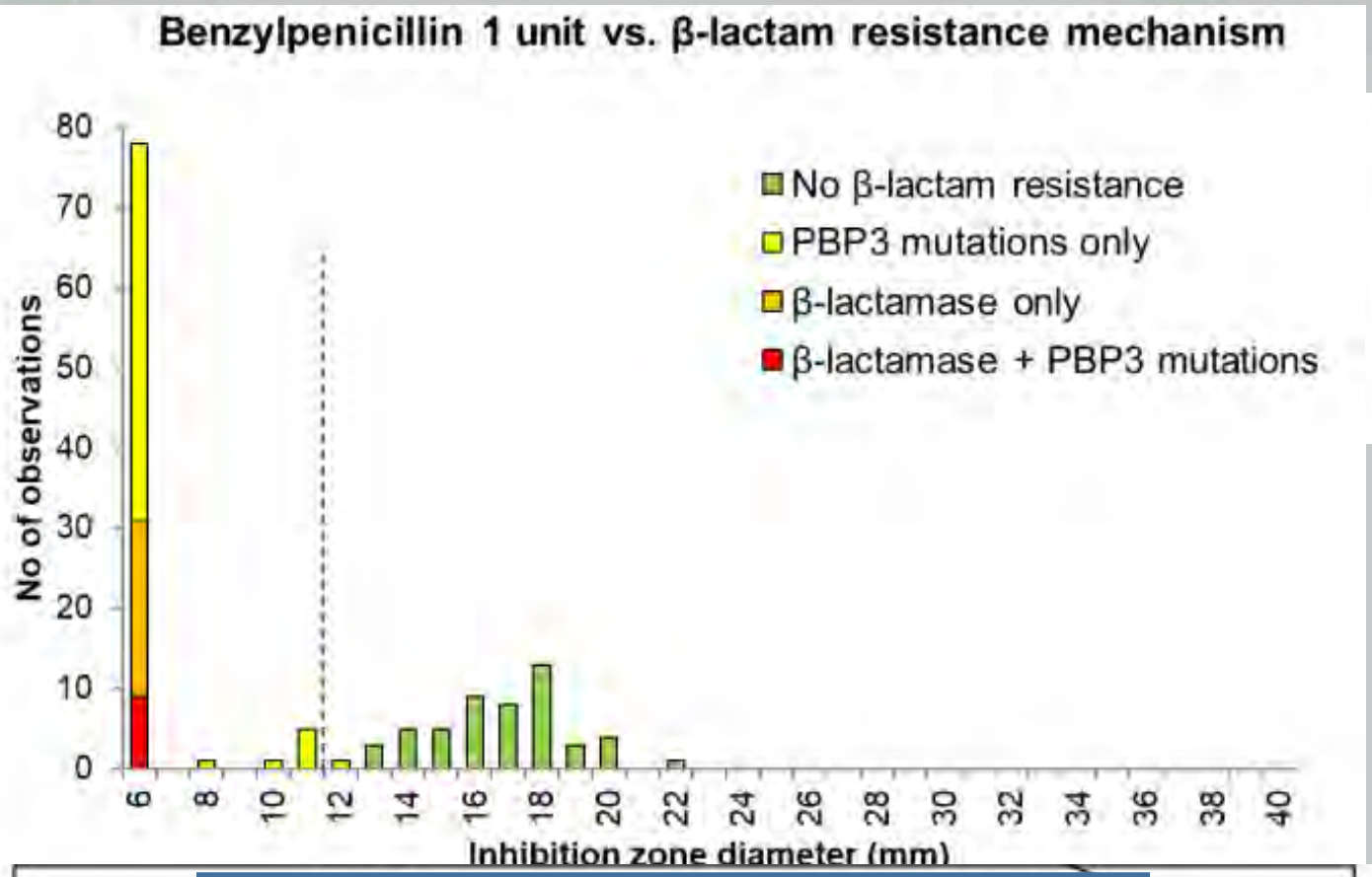
Ampicillin 2 µg disk diffusion per beta-lactam resistance mechanism

Ampicillin NOT sensitive enough to pick up low level resistant BLNAR strains



- Some strains of H. influenzae with PBP3 mutations have ampicillin MICs as low as 0.5 mg/L
- many others group around the susceptibility breakpoint (i.e. ≤1 mg/L).
- Gradient strips can underestimate the MIC

Benzylpenicillin screening disk by EUCAST



PCG 1 unit zone diameter ≥12 mm

Mechanism: excludes all beta-lactam resistance mechanisms

Report susceptible **(S)** to beta-lactam agents for which clinical breakpoints are available, including those with "Note", and those with meningitis breakpoints. **Exception:** Oral amoxicillin, oral amoxicillin-clavulanic acid and oral cefuroxime are reported "susceptible, increased exposure" **(I).**

No further testing required.

PCG 1 unit zone diameter <12 mm

Mechanism: beta-lactamase and/or PBP3 mutations

Further testing: test for beta-lactamase.

In meningitis, determine the MIC for the agent considered for clinical use and interpret according to the clinical breakpoints.

Beta-lactamase negative

Mechanism: PBP3 mutations

Perform susceptibility testing for the relevant agents and interpret according to breakpoints.

For cefepime, cefpodoxime and imipenem, if PCG 1 unit <12 mm and susceptible by agent disk diffusion test, determine the MIC of the agent and interpret according to the clinical breakpoints.

Abx	ST result
Ampicillin IV	R
Augmentin IV	R
Cefuroxime IV	R
Cefuroxime/amp/AUG Po	R

Abx	ST result
Ampicillin IV	R
Augmentin IV	S
Cefuroxime IV	S
CXM/AUG Po	S, ↑Exp

Beta-lactamase positive

Mechanisms: beta-lactamase with or without PBP3 mutations

Report resistant **(R)** to ampicillin, amoxicillin and piperacillin (without beta-lactamase inhibitor).

For other beta-lactam agents, read the amoxicillin-clavulanic acid 2-1 µg disk and interpret as below.

Abx	ST result
Ampicillin IV	R
Augmentin IV	R
Cefuroxime IV	R
Cefuroxime/amp/AUG Po	R

Amoxicillin-clavulanic acid 2-1 µg ≥15 mm

Mechanism: beta-lactamase only

Report susceptible **(S)** to agents (other than ampicillin, amoxicillin and piperacillin) for which clinical breakpoints are available, including those with "Note", and those with meningitis breakpoints.
Exception: Oral amoxicillin-clavulanic acid and oral cefuroxime are reported "susceptible, increased exposure" **(I)**.

Amoxicillin-clavulanic acid 2-1 µg <15 mm

Mechanisms: beta-lactamase **and** PBP3 mutations

Perform susceptibility testing for the relevant agents and interpret according to breakpoints.

For cefepime, cefpodoxime and imipenem, if PCG 1 unit <12 mm and susceptible by agent disk diffusion test, determine the MIC of the agent and interpret according to the clinical breakpoints.

Penicillins[1]	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)		
	S ≤	R >	ATU		S ≥	R <	ATU
Benzylpenicillin	IE	IE			IE	IE	
Benzylpenicillin (screen only)[1]	NA	NA		1 unit	12[A,B]	12[A,B]	
Ampicillin (indications other than meningitis)[2]	1	1		2	18[A,B]	18[A,B]	
Ampicillin (meningitis)[2]	IE	IE			IE	IE	
Ampicillin-sulbactam	1[3,4]	1[3,4]			Note[A,D]	Note[A,D]	
Amoxicillin iv (indications other than meningitis)[2]	2	2			Note[A,E]	Note[A,E]	
Amoxicillin iv (meningitis)[2]	IE	IE			IE	IE	
Amoxicillin oral[2]	0.001	2			Note[A,F]	Note[A,F]	
Amoxicillin-clavulanic acid iv	2[5]	2[5]		2-1	15[A,B]	15[A,B]	
Amoxicillin-clavulanic acid oral	0.001[5]	2[5]		2-1	50[A,B]	15[A,B]	
Piperacillin[2]	IE	IE			IE	IE	
Piperacillin-tazobactam	0.25[6]	0.25[6]		30-6	27[A,B]	27[A,B]	26-28[B,C]

PO augmentin→S, increased exposure

S.pneumoniae:
Warning on Gradient test on underestimation of penicillin MIC compared to reference method

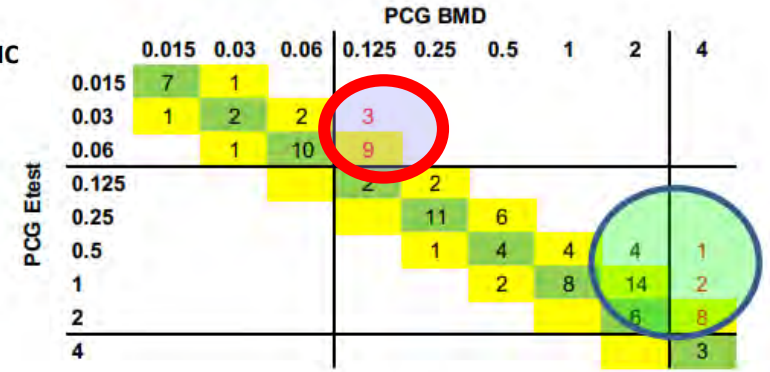
Following questions from NEQAS, EARS-Net and EUCAST users, the EDL investigated the accuracy of benzylpenicillin gradient tests (Etest™, bioMerieux; MTS™, Liofilchem). Both gradient tests were tested on in-house prepared MH-F agar from Oxoid (Thermo Fisher Scientific) och BBL (BD). Broth microdilution using Mueller-Hinton-F (MH-F) broth was used as the reference.

Both gradient tests were found to frequently underestimate MIC values by one or more doubling dilutions. In the area around the R breakpoint (0.5 – 4 mg/L), and with some variation between the MH-F media and the two tests, 0 – 37% of values were on the reference MIC, 63 – 100 % were below and 0-10 % of the values above the reference MIC.

Conclusion: Available gradient tests (Etest™ and MTS™) systematically underestimate benzylpenicillin MIC values in *S. pneumoniae*. This is especially detrimental in the important area close to the R breakpoint.

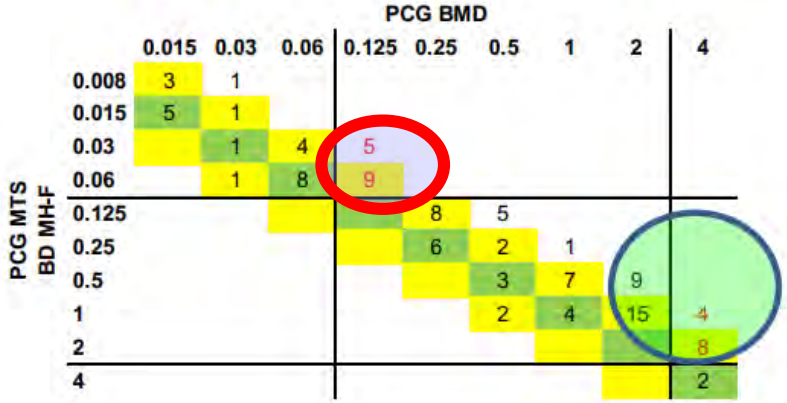
The bias unfortunately occur **near the breakpoint**

Etest



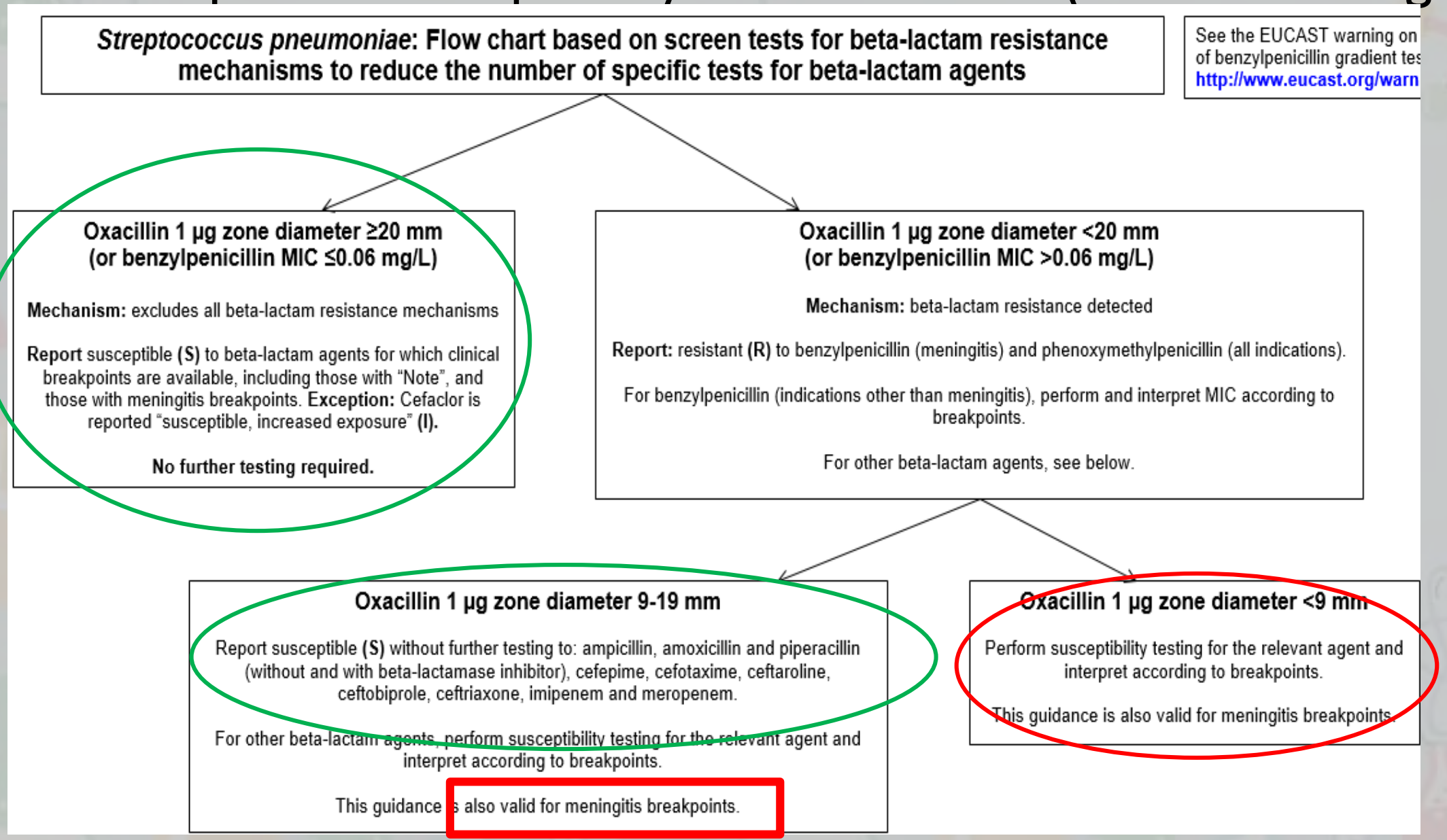
>2 dilutions lower	1
2 dilutions lower	9
1 dilution lower	46
Identical	53
1 dilution higher	5
2 dilutions higher	
>2 dilutions higher	

MTS

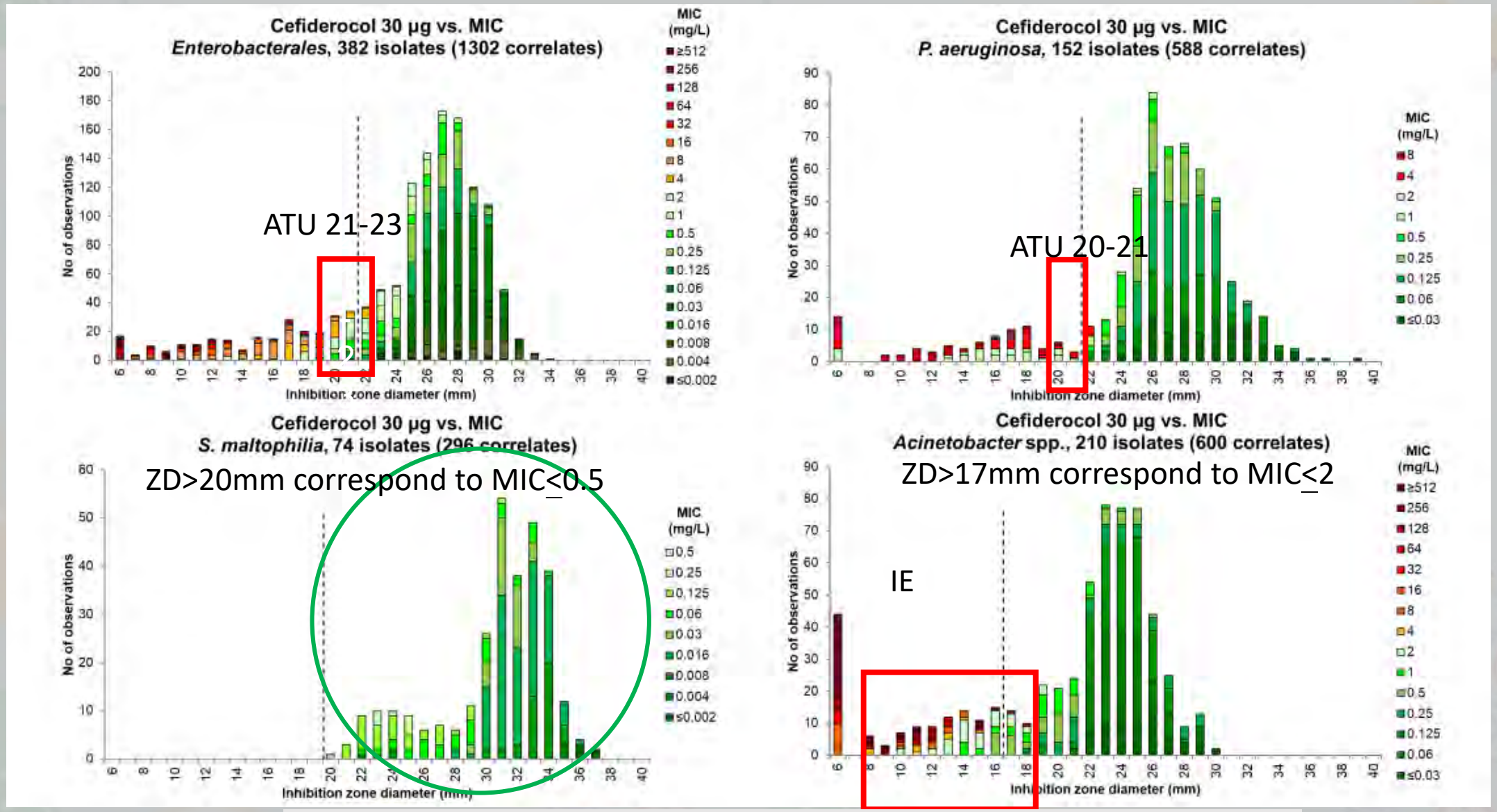


>2 dilutions lower	
2 dilutions lower	25
1 dilution lower	57
Identical	29
1 dilution higher	3
2 dilutions higher	
>2 dilutions higher	

Streptococcus pneumoniae:
OXA disc predict susceptibility of beta-lactams (include meningitis)



Cefiderocol-DDT standardized unsupplemented Mueller–Hinton agar plates



Downgrade the ST category in ATU: This drug is usually the last resort

Topical agents
Screening cut-off values for detection of phenotypic resistance

In the absence of clinical data on outcome related to MIC of infecting organisms, EUCAST has not been able to determine relevant clinical breakpoints for topical use of antimicrobial agents. Laboratories are advised to either use the regular breakpoints or the cut-off values listed below to distinguish between organisms without and with acquired resistance mechanisms (**for further details see EUCAST Guidance Document on www.eucast.org**). When reporting the susceptibility of agents for topical use, clarify that results refer to topical use only.

Organisms	**Screening cut-off values for the detection and reporting of phenotypic resistance.** Report resistant (R) for isolates with MIC above or inhibition zone diameter below the cut-off value. Otherwise report susceptible (S).		Gentamicin	Tobramycin	Pefloxacin (screen only)[1]	Norfloxacin (screen only)[1]	Nalidixic acid (screen only)[1]	Ciprofloxacin	Levofloxacin	Ofloxacin	Chloramphenicol	Colistin (for polymyxin B)	Fusidic acid	Neomycin (framycetin)	Bacitracin	Mupirocin	Retapamulin
	Disk content	(µg)	10	10	5	10	30	5	5	5	30	-	10	10	-	200	-
Enterobacterales	**MIC**	(mg/L)	2	2	-	-	-	0.125	0.25	0.25	16	2	-	8	-	-	-
	Zone diameter	(mm)	17	16	24	-	-	Note[1]	Note[1]	Note[1]	17	-	-	12	-	-	-
P. aeruginosa	**MIC**	(mg/L)	8	2	-	-	-	0.5	2	2	ND	4	-	ND	-	-	-
	Zone diameter	(mm)	15	18	-	-	-	26	18	ND	ND	-	-	ND	-	-	-
***Acinetobacter* spp.**	**MIC**	(mg/L)	4	4	-	-	-	1	0.5	1	ND	2	-	ND	-	-	-
	Zone diameter	(mm)	17	17	-	-	-	21	23	ND	ND	-	-	ND	-	-	-
S. aureus	**MIC**	(mg/L)	2	2	-	-	-	1	0.5	1	16	-	0.5	1	ND	1[2]	0.5
	Zone diameter	(mm)	18	18	-	17	-	Note[1]	Note[1]	Note[1]	18	-	24	14	ND	30[2]	ND
S. pneumoniae	**MIC**	(mg/L)	-	-	-	-	-	4	2	4	8	-	ND	-	ND	-	-
	Zone diameter	(mm)	-	-	-	10	-	Note[1]	Note[1]	Note[1]	21	-	ND	-	ND	-	-
Streptococcus groups A, B, C and G	**MIC**	(mg/L)	-	-	-	-	-	2	2	4	8	-	32	-	ND	0.5	0.125
	Zone diameter	(mm)	-	-	-	12	-	Note[1]	Note[1]	Note[1]	21	-	ND	-	ND	ND	ND
H. influenzae	**MIC**	(mg/L)	4	8	-	-	-	0.06	0.06	0.06	2	-	ND	ND	-	-	-
	Zone diameter	(mm)	ND	ND	-	-	23	Note[1]	Note[1]	Note[1]	28	-	ND	ND	-	-	-
M. catarrhalis	**MIC**	(mg/L)	ND	ND	-	-	-	0.125	0.125	0.25	2	-	ND	ND	-	-	-
	Zone diameter	(mm)	ND	ND	-	-	23	Note[1]	Note[1]	Note[1]	31	-	ND	ND	-	-	-

Notes

1. Screening agent for detection of fluoroquinolone resistance (pefloxacin for *Enterobacterales*, norfloxacin for Gram-positive organisms and nalidixic acid for *H. influenzae* and *M. catarrhalis)*.
2. Breakpoints for nasal decontamination S ≤1, R >256 mg/L (S ≥30, R <18 mm for the mupirocin 200 µg disk). Isolates in the I category are associated with short term suppression (useful preoperatively) but, unlike fully susceptible isolates, long term eradication rates are low.

ND = No ECOFF available.

What to do if there is no breakpoint

- Disk diffusion test NOT interpretable
- Do MIC with a reliable method
  - Gradient tests can only be relied on when validated for the species and agent, either by the manufacturer or by the user, and with simultaneous QC
  - A gradient test developed and validated for one species cannot automatically be trusted with another species.
- Use ECOFF finder to infer whether the drug is likely/unlikely to be effective
  - If above ECOFF, less likely to be effective
  - If below ECOFF, may/may not be effective

MIC distribution data and ECOFF finder

Method ● **MIC** ○ **Disk diffusion**

Antimicrobial

Antimicrobial ...

Species

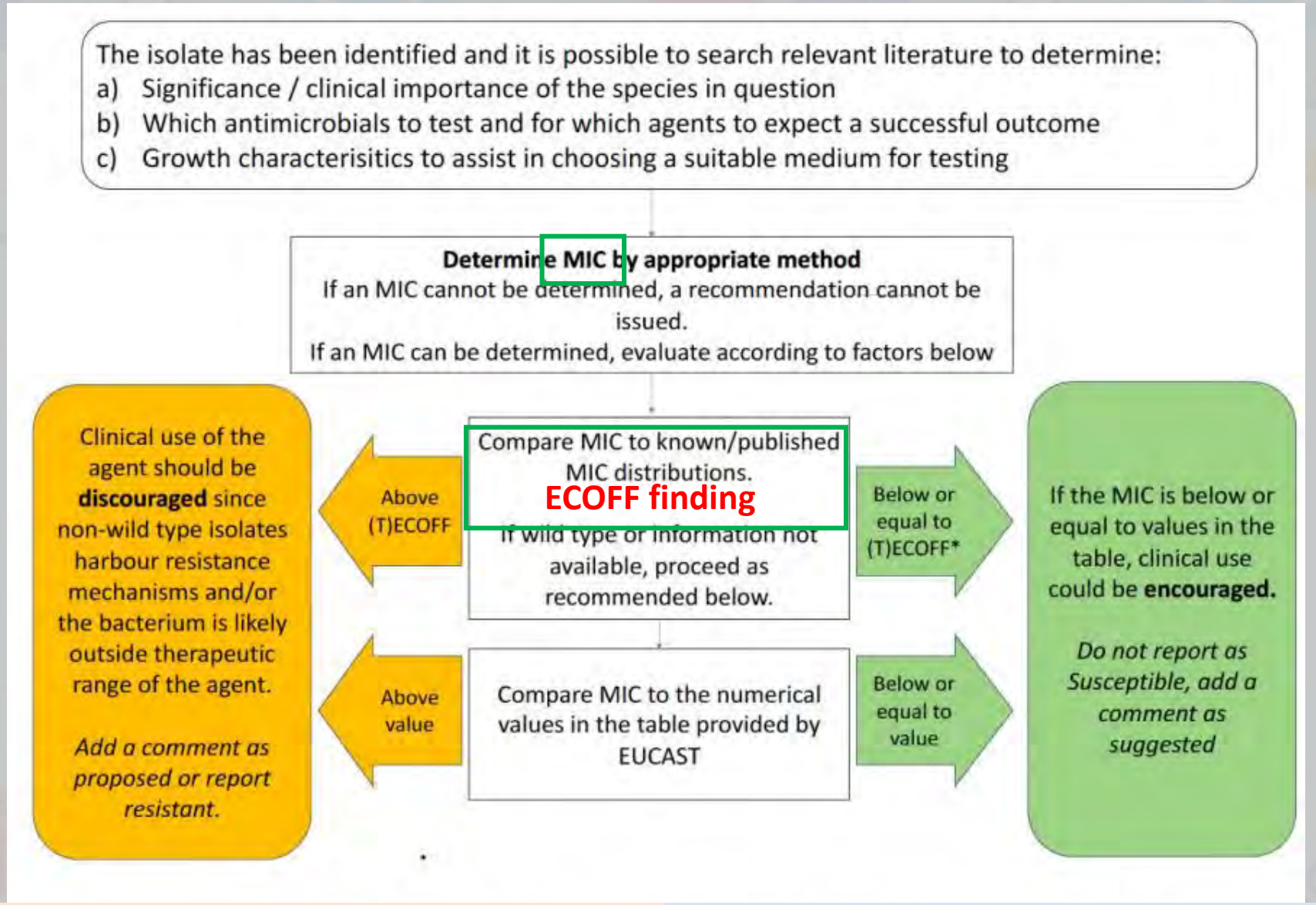
Campylobacter jejuni

Elements per page 50

MIC distributions for Campylobacter jejuni, 2024-02-21

Species: Campylobacter jejuni (Method: MIC)

	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	Distributions	Observations	(T)ECOFF	Confidence interval
Amoxicillin	0	0	0	0	0	0	0	1	1	17	27	89	135	19	40	72	0	0	0	5	401	16	16 - 64
Ampicillin	0	0	0	0	0	0	2	4	26	59	122	122	48	19	26	12	1	3	0	6	444	16	4 - 32
Azithromycin	0	0	0	2052	9805	9891	3888	1176	186	62	12	14	10	2	5	7	276	15	85	41	27486	0.25	0.125 - 0.25
Chloramphenicol	0	0	0	7	4	8	33	131	634	1432	1666	806	243	71	18	2	0	0	1	25	5056	16	4 - 16
Ciprofloxacin	1	2	12	194	1795	10023	9570	1844	201	60	50	334	2494	1539	756	593	35	0	0	58	29503	0.5	0.125 - 0.25
Clindamycin	0	0	0	15	789	5911	11717	6599	1810	421	137	109	91	53	76	13	1	2	12	43	27756	0.5	0.25 - 1
Doxycycline	0	0	0	0	12	59	75	18	10	6	17	21	52	53	20	0	0	0	0	3	343	(0.5)	0.016 - 4
Ertapenem	6	38	70	101	71	59	6	18	0	2	2	0	0	0	0	0	0	0	0	4	373	(0.125)	0.125 - 0.5
Erythromycin	0	0	0	2	23	171	2337	10772	10000	4828	1421	257	34	16	5	29	256	16	90	64	30257	4	4 - 16
Florfenicol	0	0	0	0	16	38	53	177	6249	14305	2094	254	30	6	1	0	0	0	0	27	23223	4	1 - 4
Gentamicin	0	0	0	2	37	156	1754	6926	15430	3062	114	12	5	0	5	62	0	0	1	50	27566	2	0.5 - 2
Imipenem	0	0	0	2	33	87	53	20	3	1	0	0	0	0	0	0	0	0	0	1	199	ID	
Kanamycin	0	0	0	0	0	0	0	0	0	9	56	106	21	2	0	0	0	5	0	1	199	ID	
Levofloxacin	0	0	0	0	0	1	9	16	5	3	0	1	3	1	1	3	0	0	0	1	43	ID	



Formal categorising of the susceptibility of the organism is not possible. The MIC suggests that the agent should not be used for therapy".

"Formal categorising of the susceptibility of the organism is not possible. A cautious interpretation suggests that the agent may be considered for therapy."

Aerobic organisms

Agents and notes for aerobic bacteria	MIC-values above which therapy with the agent should be discouraged	Notes
Benzylpenicillin	0.25	The value is valid unless beta-lactamase production is suspected.
Ampicillin, Amoxicillin, Ampicillin-sulbactam, Amoxicillin-clavulanic acid (IV only), Gram-negative organisms	2	If a beta-lactamase is detected, the value is only valid for amoxicillin-clavulanic acid and ampicillin-sulbactam.
Ampicillin, Amoxicillin, Ampicillin-sulbactam, Amoxicillin-clavulanic acid (IV only), Gram-positive organisms	0.5	If a beta-lactamase is detected, the value is only valid for amoxicillin-clavulanic acid and ampicillin-sulbactam.
Piperacillin-tazobactam, Gram-negative organisms	2	The piperacillin-tazobactam breakpoint is conservative and on par with the aminopenicillin breakpoints
Cefotaxime	0.25	Cefotaxime and ceftriaxone – resistance to either excludes the use of both.
Ceftriaxone	0.25	Cefotaxime and ceftriaxone – resistance to either excludes the use of both.
Imipenem	2	Species specific breakpoints are often 2 mg/L.
Meropenem	2	Species specific breakpoints are 0.25 – 2 mg/L
Ciprofloxacin	0.25	Species specific breakpoints are 0.25 – 1 mg/L.
Moxifloxacin	0.25	Species specific breakpoints are 0.125 – 0.5 mg/L
Clindamycin, Gram-positive organisms	0.5	Species specific breakpoints are 0.25 – 0.5 mg/L.
Tetracycline (report doxycycline, minocycline)	2	Tetracycline (as a representative for tetracycline, doxycycline, and minocycline) species specific breakpoints are 0.5 – 2 mg/L.
Tigecycline	0.5	Species specific breakpoints are 0.125 – 0.5 mg/L.
Rifampicin, Gram-positive organisms	0.125	Species specific breakpoints are 0.06 – 0.125 mg/L.
Linezolid, Gram-positive organisms	2	Species specific breakpoints are 2 - 4 mg/L
Vancomycin, Gram-positive organisms	2	Species specific breakpoints are 2 mg/L.
Dalbavancin, Gram-positive organisms	0.125	Species specific breakpoints are 0.125 mg/L.
Trimethoprim-sulfamethoxazole	2	Species specific breakpoints are 0.5 – 2 mg/L.

Anaerobic organisms

Agents and notes for anaerobic bacteria	MIC-values above which therapy with the agent should be discouraged	
Benzylpenicillin	0.5	Breakpoints for other anaerobic bacteria are 0.06 – 0.5 mg/L. The value is valid unless beta-lactamase production is suspected.
Amoxicillin	0.5	Breakpoints for other anaerobic bacteria are 0.25 – 0.5 mg/L. The value is valid unless beta-lactamase production is suspected.
Amoxicillin-clavulanic acid	0.5	Breakpoints for other anaerobic bacteria are 0.25 – 0.5 mg/L.
Ampicillin-sulbactam	0.5	Breakpoints for other anaerobic bacteria are 0.25 – 0.5 mg/L.
Piperacillin-tazobactam	2	Breakpoints for other anaerobic bacteria are 0.5 – 2 mg/L.
Meropenem	1	Breakpoints for other anaerobic bacteria are 0.03 – 1 mg/L.
Imipenem	1	Breakpoints in other anaerobic bacteria are 0.03 – 1 mg/L
Ertapenem	0.25	Breakpoints in other anaerobic bacteria are 0.06 – 0.5 mg/L
Clindamycin	0.5	Breakpoints for other anaerobic bacteria are 0.25 mg/L.
Metronidazole	4	Breakpoints for other anaerobic bacteria are 0.5 - 4 mg/L.
Vancomycin	2	Only relevant for a few gram-positive anaerobic bacteria. A breakpoint of 2 mg/L is common for targeted species.
Linezolid (Gram-positive)	2	Breakpoints for *Cutibacterium acnes* is 2 mg/L.
Rifampicin (Gram-positive)	0.125	Breakpoints for most species already in the EUCAST breakpoint tables are 0.06 – 0.125 mg/L.
Moxifloxacin (mixed infections)	1	Moxifloxacin has been used in the treatment of mixed anaerobic infections but not for targeted therapy. Breakpoints for other species with breakpoints are 0.125 – 0.5 mg/L. MIC-values for different anaerobic species are usually 0.5 – 2 mg/L.

The proposed values are based

1. a compromise between current EUCAST susceptible (S or I) breakpoints for species already in the tables
2. wild type distributions for microorganisms when available
3. PK/PD breakpoint

Conclusion

- EUCAST provide well-validated breakpoints for AST
- Different interpretation for “I” in EUCAST from CLSI (susceptible; increased exposure)
  - Many organisms only with “S, increased exposure” breakpoint
- ATU provide technical buffer to avoid VME.
  - Test with alternative method or just downgrade the ST category
- BMD is the gold standard MIC testing method in EUCAST, with considerable technical variability
- Commercials/Gradient strips needs to be validated to be reliable. Discrepancy/bias with BMD is common
  - Disk diffusion test is a very reliable test, DO-NOT be over-confidence on MIC result
- Disk diffusion breakpoint available for common anaerobes
- Disk diffusion screening test for many organisms for discriminating S and R
- Strict and more frequent QC requirement for EUCAST
- ECOFF finders available to help interpret ST result for those organisms without breakpoint