

Area of Technical Uncertainty (ATU)

How to deal with ATUs in the clinical
laboratory?

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Gothenburg 28 May 2019

For 2019 EUCAST decided

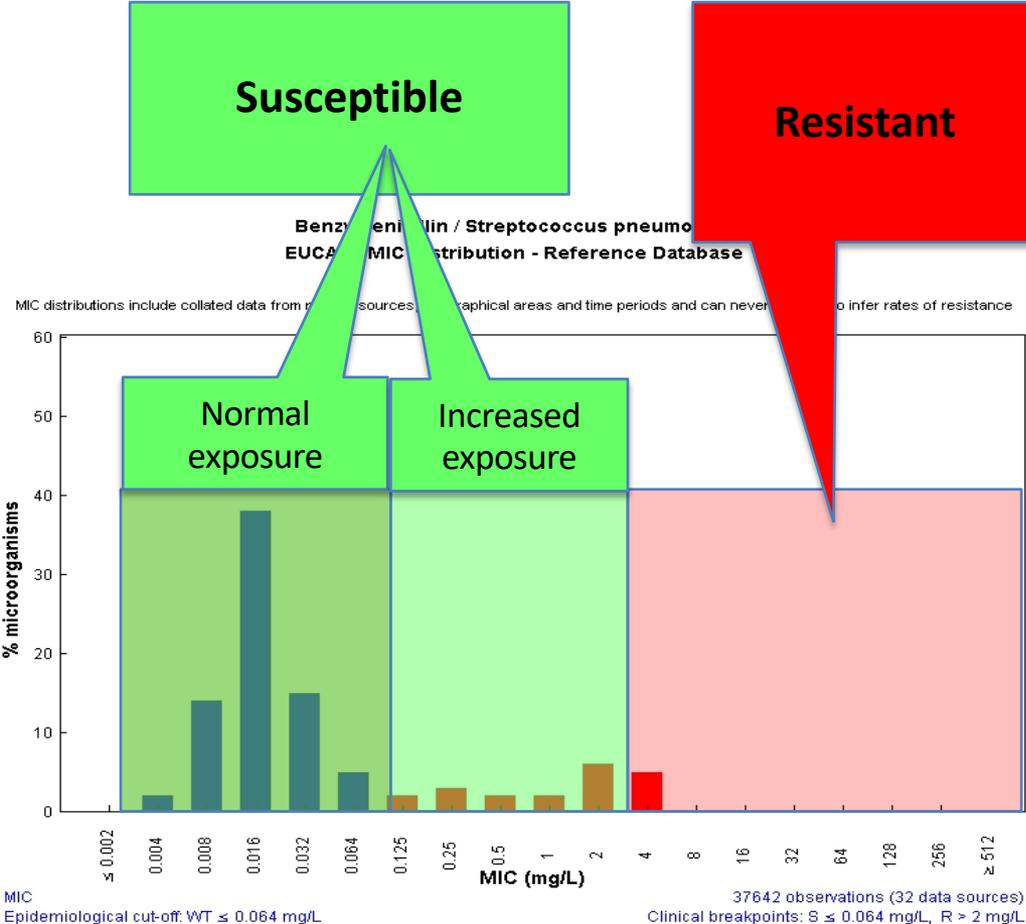
- To change the definitions of S, I and R
- To retain the letters S, I och R
- To emphasize the relationship between the concentration of the antimicrobial agent at the site of the infection AND the breakpoints for categorisation (S, I and R)
- To task clinical laboratories with the responsibility for uncertain laboratory results, irrespective of origin

The new definitions of S, I and R

- 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

SIR – new definitions 2019



New definitions of S, I and R and the introduction of ATU

- In breakpoint table 9.0 (2019), EUCAST introduced new definitions of S, I and R, emphasized the relationship between breakpoints and antimicrobial exposure (“All breakpoints are dose dependent!”) and divided the responsibility for susceptibility testing between

SHE ?

S^{HE} and I – is there a difference?

- No – only a delay in decisions.
- Some S^{HE} were changed to “I” already in the 2019 table. Others (those with S^{HE} in the 2019 table) were deemed in need of further consultation (ongoing)
- The plan is for S^{HE} to disappear in 2020 and to be replaced by “Susceptible, increased exposure”
- The consultation is on the EUCAST website – it is public, and anyone who has good arguments against the change should respond to the consultation

[Resistance mechanisms](#)

[Guidance documents](#)

[Consultations - New!](#)

[MIC and zone distributions and ECOFFs](#)

[AST of bacteria](#)

[AST of mycobacteria](#)

[AST of fungi](#)

[AST of veterinary pathogens](#)

[Frequently Asked Questions \(FAQ\)](#)

[Meetings](#)

[Presentations and statistics](#)

[Warnings!](#)

Documents

[Rationale Documents](#)

[Standard Operation Procedures](#)

[Discussion documents](#)

[Consultations](#)

[Publications in journals](#)

[Technical notes](#)

[Posters](#)

[Other Documents](#)

[External documents](#)

[Reports](#)

EUCAST Consultations

Current consultations ([download form for comments](#))

- EUCAST [proposed breakpoints for *Burkholderia pseudomallei*](#).
Consultation 23 May - 30 June, 2019.
Use form ([download form](#)) to submit comments to [Gunnar Kahlmeter](#)
- CLSI/EUCAST consultation on "[Procedure for optimizing disk contents \(potencies\) for disk diffusion testing](#)".
This document has also been submitted to the CLSI meeting 16-18 June 2019".
Comments from the CLSI meeting and the EUCAST consultation will be summarised through the EUCAST consultation document.
Consultation 21 May - 31 July, 2019.
Use this form for comments ([Download form](#)) and send to [Erika Matuschek](#)
- EUCAST proposes to [Revise aminoglycoside breakpoints](#).
Consultation 17 May - 30 June, 2019.
Use the form for comments ([Download form](#)) to send to [John Turnidge](#).
- EUCAST AMST: Reference method for the determination of MICs in *Mycobacterium tuberculosis* complex.
Consultation 15 May - 26 June, 2019.
[Rationale for the proposed broth microdilution MIC method in *M. tuberculosis* complex.](#)
[Technical protocol for broth microdilution in *M. tuberculosis* complex.](#)
[Microtiter plate outline.](#)
[Summary of development test results.](#)
Use the form for comments ([Download form](#)) to send comments to AMST scientific secretary [Thomas Schön](#).
- [EUCAST proposes that agents and species currently categorised as Susceptible^{HE} \(S^{HE}\) are re-categorised as "Susceptible, increased exposure" \(I\).](#)
Consultation 9 May - 23 June, 2019.
Send comments to [Gunnar.Kahlmeter\[at\]eucast.org](mailto:Gunnar.Kahlmeter[at]eucast.org). [Download form for comments](#).
- Other consultations expected during 2019: revision of expert rules.
[Download form for comments](#).

Table 1a. Current “Susceptible^{HE}” agents/species and breakpoints and proposed new breakpoints categorizing wild type distributions as “Susceptible increased exposure”.

Species	Agent	Current breakpoint with a comment to use high dose	Proposed new breakpoint* to place wild type isolates in the I-category.	New susceptibility category of wild type isolates
<i>Enterobacterales</i>	Cefuroxime IV	8/8	0.001/8	I
<i>P. aeruginosa</i>	Piperacillin-tazobactam	16/16	0.001/8	I
	Ticarcillin	16/16	0.001/8	I
	Cefepime	8/8	0.001/8	I
	Ceftazidime	8/8	0.001/8	I
	Aztreonam	16/16	0.001/8 or 16	I
	Ciprofloxacin	0.5/0.5	0.001/0.5	I
	Levofloxacin	1/1	0.001/1	I
	Imipenem	4/4	0.001/4	I
<i>S.maltophilia</i>	Trimethoprim-sulfamethoxazole	4/4	0.001/2	I
<i>Staphylococcus</i>	Ciprofloxacin	1/1	0.001/1	I
	Levofloxacin	1/1	0.001/1	I
	Ofloxacin	1/1	0.001/1	I
<i>Strc. A,C,G</i>	Levofloxacin	2/2	0.001/2	I
<i>S. pneumoniae</i>	Levofloxacin	2/2	0.001/2	I
<i>H. influenzae</i>	Amoxicillin (oral)	2/2	0.001/2	I
	Amoxicillin-clavulanate (oral)	2/2	0.001/2	I
*The breakpoint $S \leq 0.001$ mg/L represents a convention introduced to prevent that occasional organisms are categorised as susceptible				

Most AST is unproblematic

...if your method is robust and QC:ed

...if your device is QC:ed, at both ends of the scale

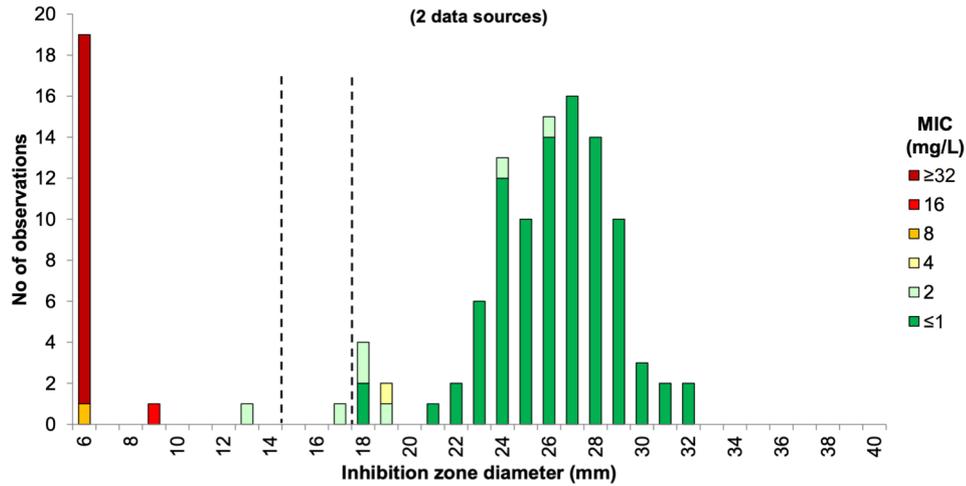
...if your disks and gradient test are high quality

...if your MH medium is dependable

...if your staff properly trained

...and the agent, species and resistance mechanisms
co-operate

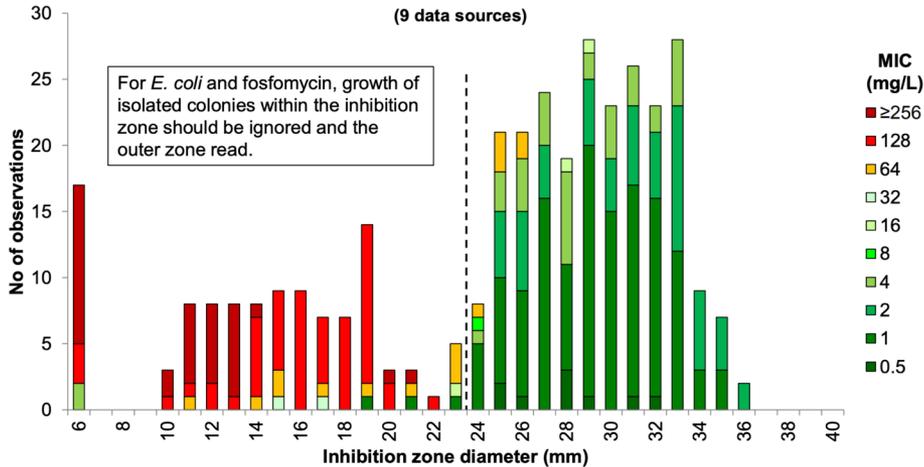
Trimethoprim 5 µg vs. MIC Enterobacteriales, 122 isolates



Breakpoints

MIC S≤2, R>4 mg/L
Zone diameter S≥18, R<15 mm

Fosfomycin 200 µg vs. MIC *E. coli*, 52 isolates (349 correlates)



Breakpoints (iv and oral)

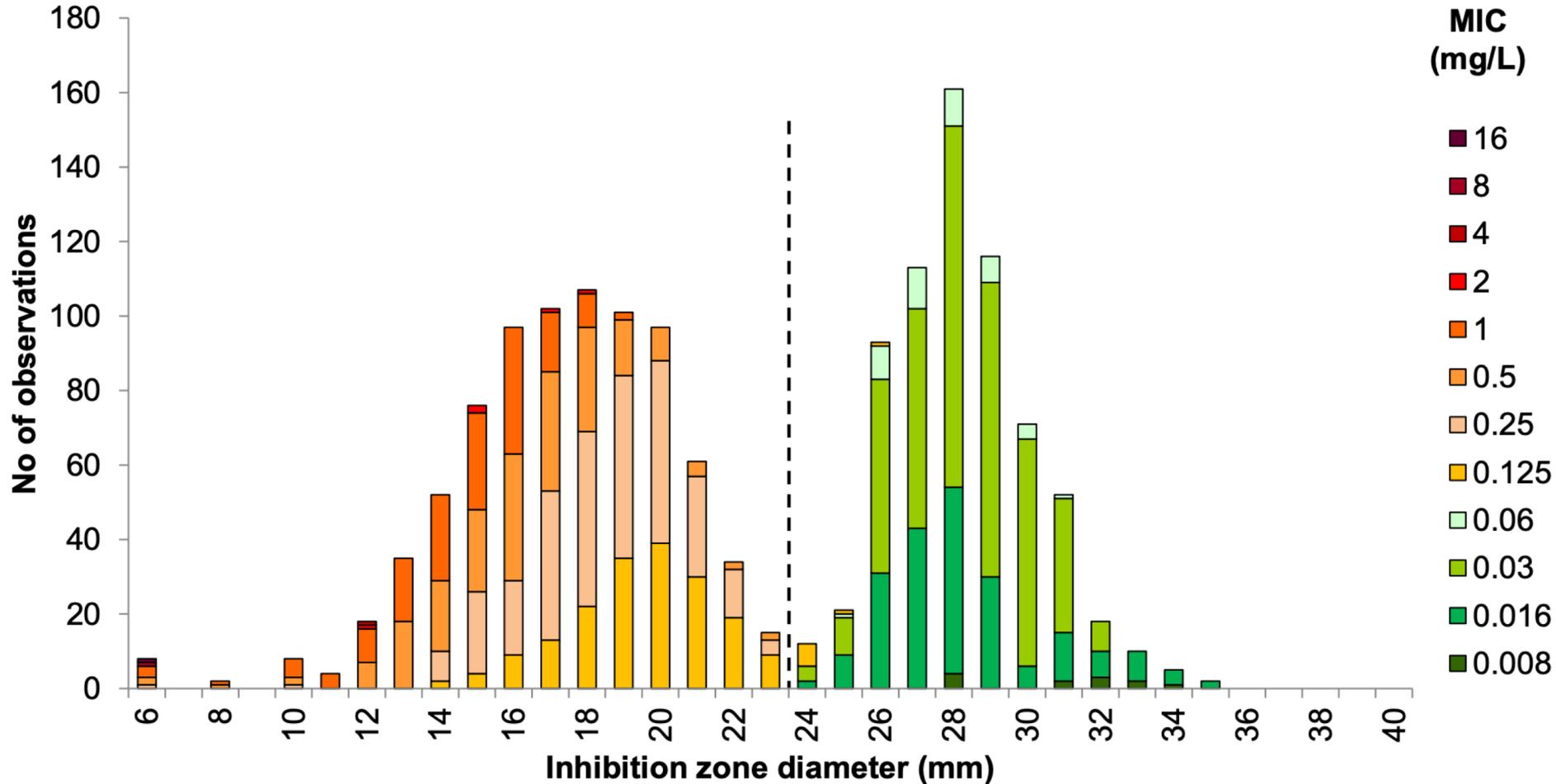
MIC S≤32, R>32 mg/L
Zone diameter S≥24, R<24 mm

ECOFF

8 mg/L

Pefloxacin 5 µg vs. Ciprofloxacin MIC

Salmonella spp., 268 isolates (1491 correlates)



Breakpoints

Ciprofloxacin MIC

$S \leq 0.06$, $R > 0.06$ mg/L

Pefloxacin zone diameter (screen)

$S \geq 24$, $R < 24$ mm

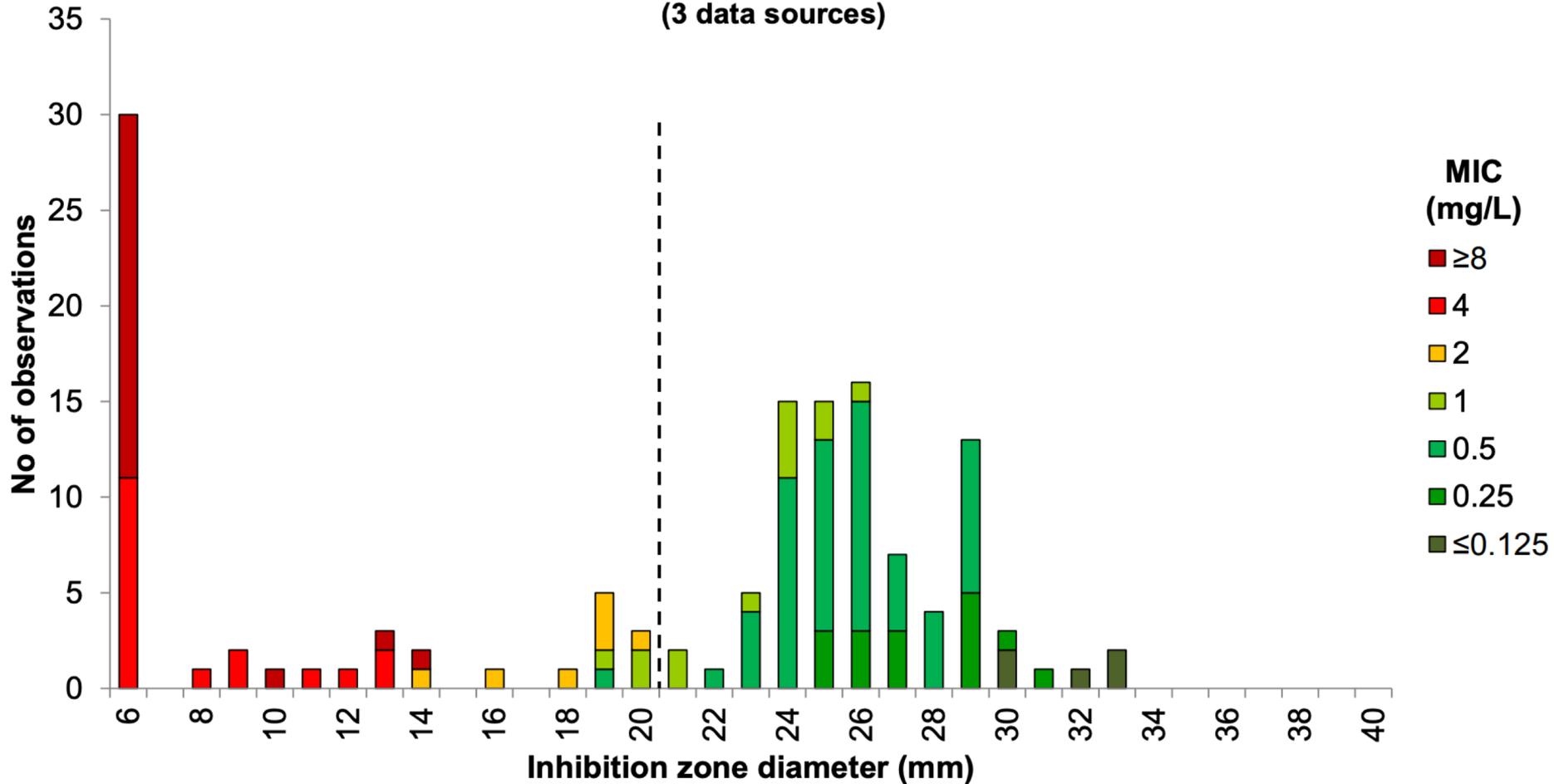
ECOFF

0.06 mg/L

Ciprofloxacin 5 µg vs. MIC

S. aureus, 136 isolates

(3 data sources)



Breakpoints

MIC S ≤ 1, R > 1 mg/L

Zone diameter S ≥ 21, R < 21 mm

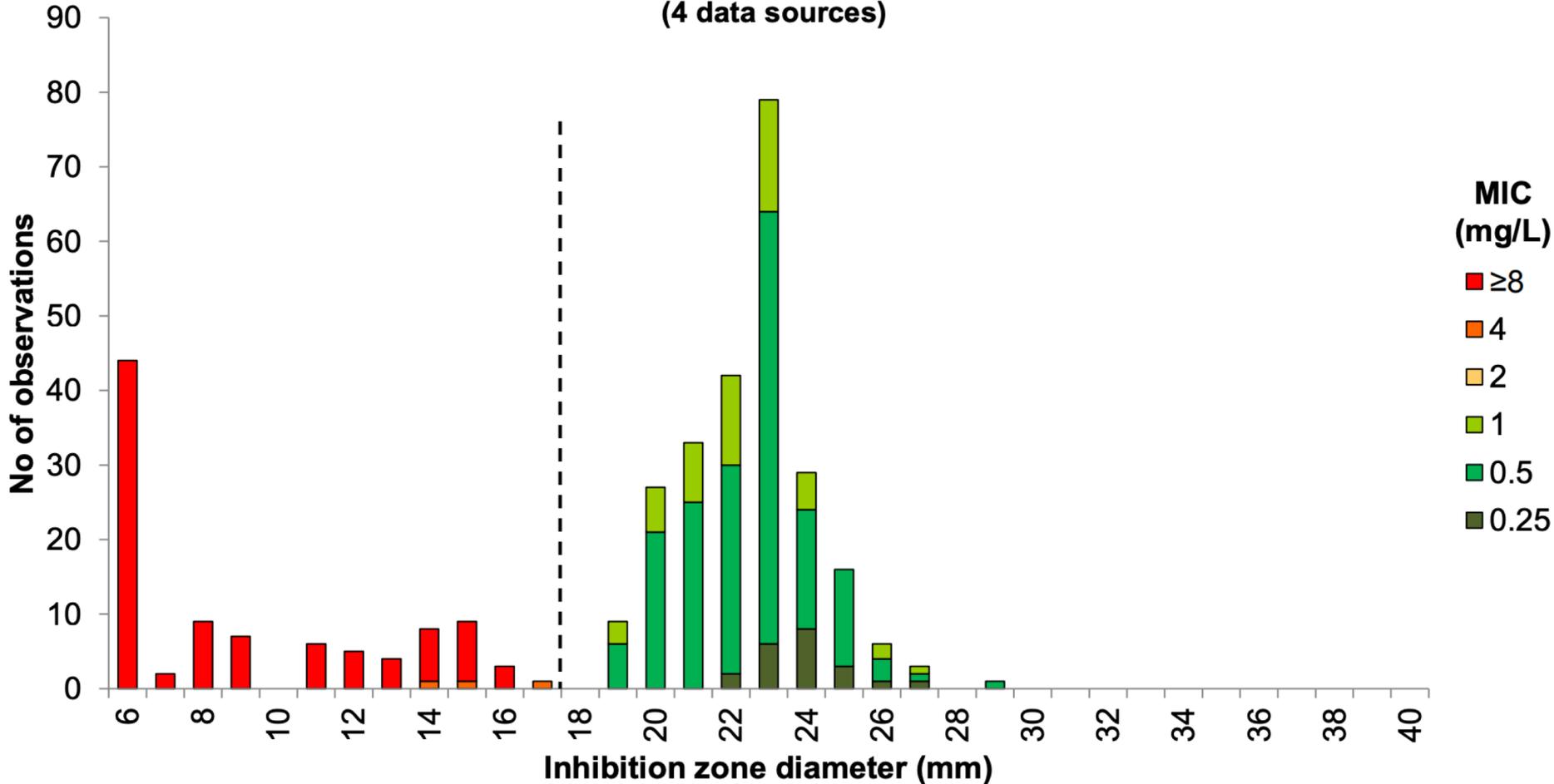
ECOFF

1 mg/L

Gentamicin 10 µg vs. MIC

S. aureus, 203 isolates (343 correlates)

(4 data sources)



Breakpoints

MIC $S \leq 1, R > 1$ mg/L

Zone diameter $S \geq 18, R < 18$ mm

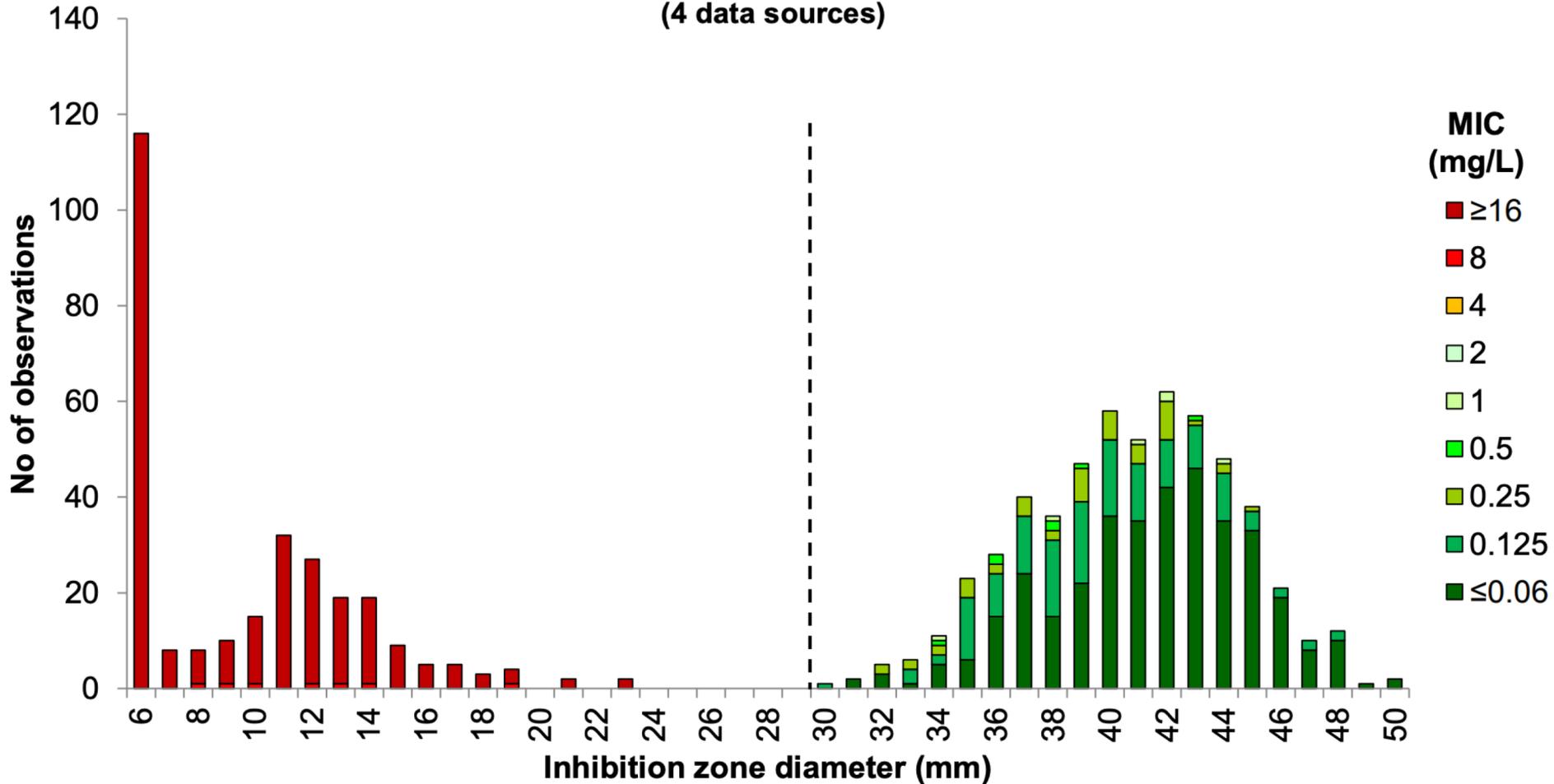
ECOFF

2 mg/L

Tetracycline 30 µg vs. MIC

C. jejuni and *coli*, 817 isolates (844 correlates)

(4 data sources)



Breakpoints

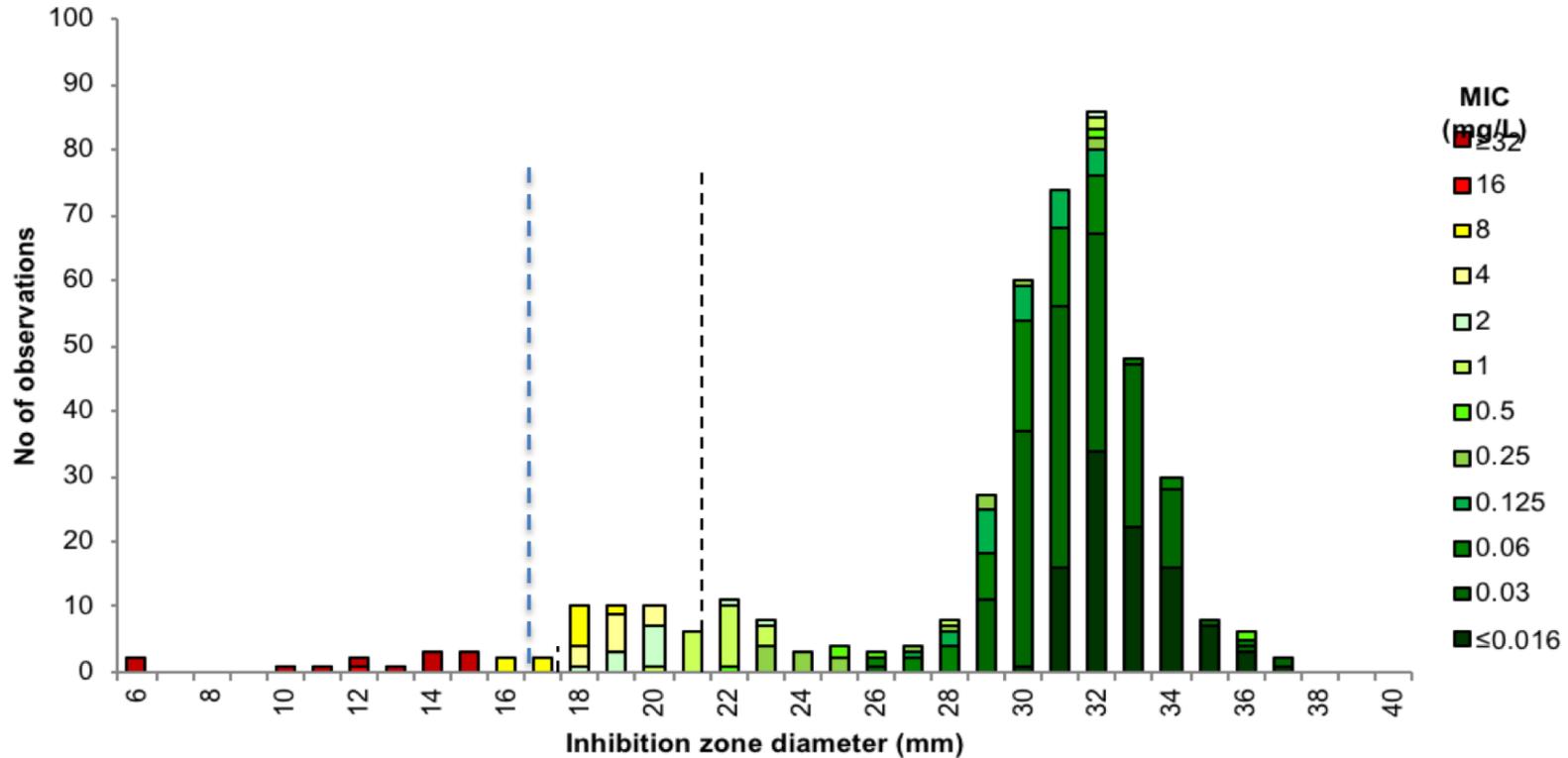
MIC S ≤ 2, R > 2 mg/L
 Zone diameter S ≥ 30, R < 30 mm

ECOFF

1 mg/L (*C. jejuni*), 2 mg/L (*C. coli*)

Meropenem and Enterobacterales – one of many examples where an ATU is not needed.

Meropenem 10 µg vs. MIC
Enterobacterales, 378 isolates (435 correlates)



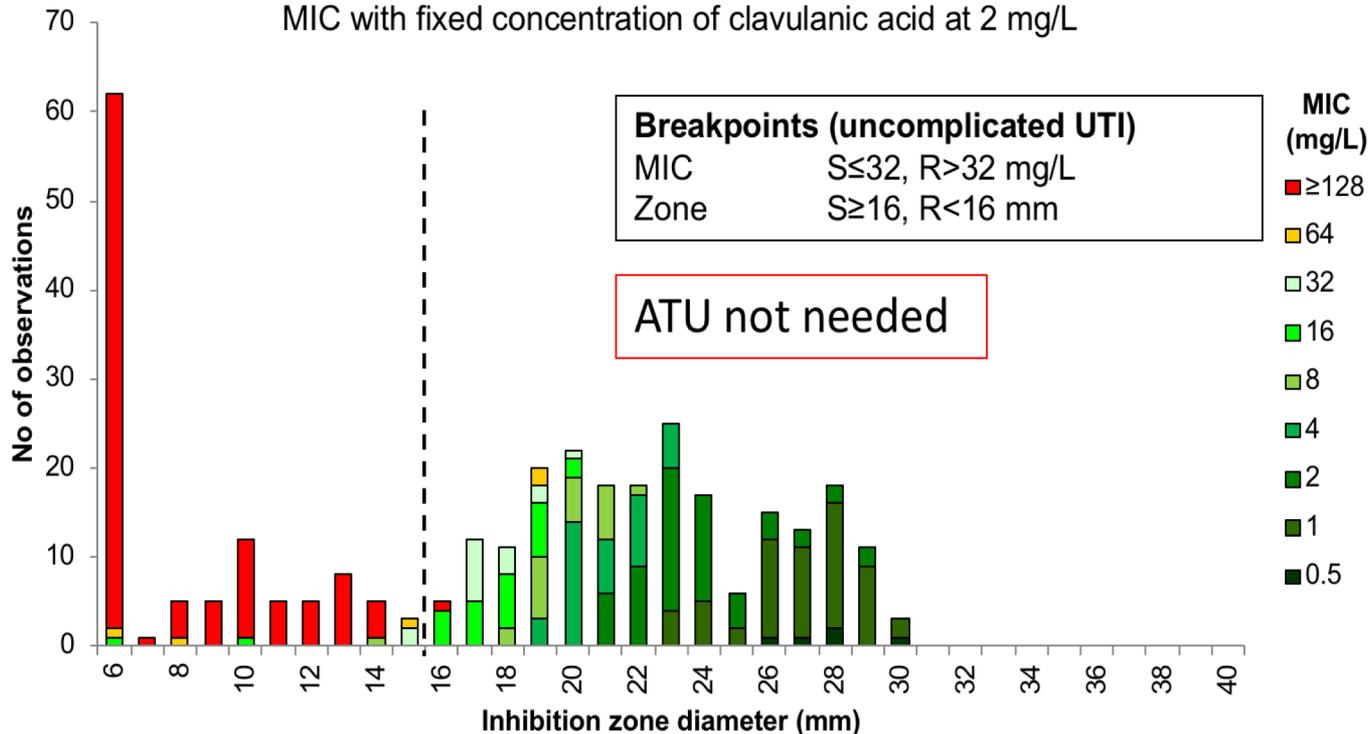
Breakpoints

MIC $S \leq 2, R > 8$ mg/L
Zone diameter $S \geq 22, R < 16$ mm

Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints for uncomplicated UTI

Amoxicillin-clavulanic acid 20-10 µg vs MIC Enterobacterales, 325 isolates

MIC with fixed concentration of clavulanic acid at 2 mg/L

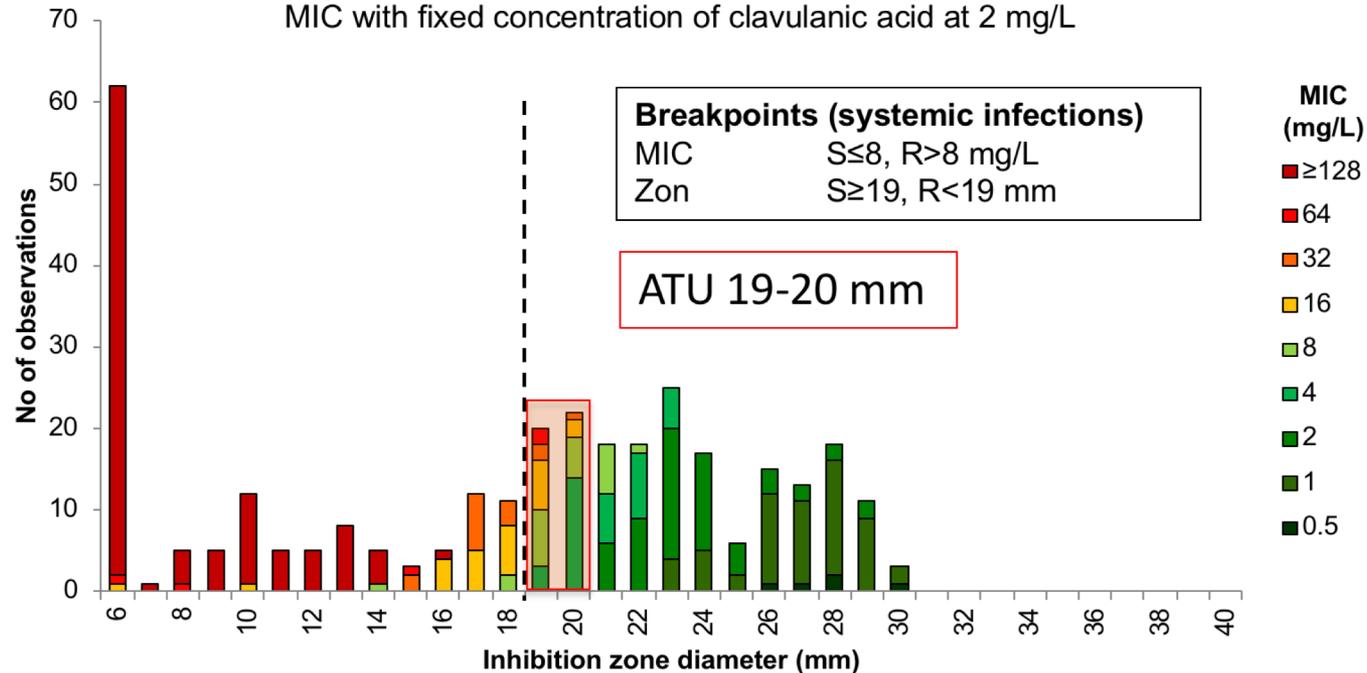


ATU defined

Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints
for systemic infections

Amoxicillin-clavulanic acid 20-10 µg vs MIC Enterobacterales, 325 isolates

MIC with fixed concentration of clavulanic acid at 2 mg/L



The ATU is a “warning” in the laboratory!

- Variation in the method which is difficult to control
 - Betalactam agents vs. *H.influenzae* with PBP3 mutations.
- Poor separation between susceptible and resistant organisms and many results close to the breakpoint (overlap).
 - Piperacillintazobactam and ciprofloxacin vs Enterobacterales
- Variation in the interpretation which is difficult to control
 - Breakpoint splits wild type (mostly avoided by EUCAST) – colistin and *Ps. aeruginosa*.
 - Breakpoint splits an important resistant population (mostly difficult to avoid) - ceftaroline and ceftobiprole in MRSA.

Area of Technical Uncertainty (ATU)

- ATU is not a fourth susceptibility category – it is to **warn laboratory staff**, not clinical colleagues, about an area where interpretation is uncertain.
- The ATU is not to compensate for poor methodological skills – on the contrary, AST today require more skills than ever before.
- ATU consists of one MIC-value or a corresponding short range of zone diameter values (2 – 3 mm).
- How the ATU is dealt with depends on the situation (the sample, the agent, the infecting organism).

Warning (ATU) – alternative actions!

- **Just repeating the test will not resolve ATU issues**
 - Tests that fail technically should always be addressed.
- **Confirm** using an alternative test – and when you do, repeat the original test. You will learn something!
- **If you do not report a result – a comment may be needed.**
 - “The result is uncertain – more testing is ongoing.”
 - “The result is uncertain – if necessary, contact the laboratory for additional testing.”
 - “Susceptibility testing of the agent is not possible.”
- **Down-grade interpretation** - to I or R.

You may have to discuss with the clinical colleague to choose the way forward, especially in serious cases where few alternatives.

Try to get an unequivocal AST result IF.....

- only few alternative antibiotics for therapy.
- in a positive blood culture (or other serious infection).
- a clinical colleague is adamant
- a frequently recurring problem

-can be easily solved.

Cases for discussion

S. epidermidis

- Pacemaker endocarditis
 - Cefoxitin-screen test 25 mm
 - What next?

S/R 25/25 mm, ATU 25 - 27

1. Perform PCR to exclude mec-genes.

**92-year old woman with recurring UTIs over the last 5 years.
Now UTI and suspected urosepsis.**

Urine sample grows *E. coli* with the following results:

Day 1:

- Ampicillin-R, Cefadroxil (Cefalexin)-R, Pivmecillinam-S, Nitrofurantoin-S, Trimethoprim-R

...you discovered that blood cultures were ongoing (so far negative), and decided to test more antibiotics:

Piperacillin-tazobactam-I (**zone diameter 18 mm***)

Cefotaxime-R

Ceftazidime-S

Ciprofloxacin-S

Trimethoprim-sulfa-R

Gentamicin-S and Tobramycin-S.

Zone diameter=I (and ATU),

MIC=unknown.

Hide PTZ for the time being - report the alternatives;

If important, perform PTZ BMD and repeat the disk test.

If not important, report PTZ-R.

*ATU 17-19 mm; ATU 16 mg/L

Day 2:

You decided to retest the isolate with MIC and disk diffusion:

Piperacillin-tazobactam (**zone diameter 17 mm, MIC 16 mg/L***)

Zone diameter=17 mm, BMD MIC=16 mg/L

You have confirmed your initial result and obtained the same category with an alternative test.

Report “Susceptible, increased exposure (I)”.

*ATU 17-19 mm; ATU 16 mg/L

H. Influenzae

- Testing is unproblematic unless the isolate has a PBP3 mutation (PcG 1U <12 mm).
- If PcG 1U is ≥ 12 mm, no further testing is needed for betalactams.
- If PcG 1U is <12 mm, the isolate may be BLA-positive and/or have a PBP3 mutation. Some betalactam agents are more problematic than others, from a testing point.

***H. influenzae* and PBP3 mutations**

PcG1U disk screen will always be positive

- ***Case 1: Child with osteomyelitis***

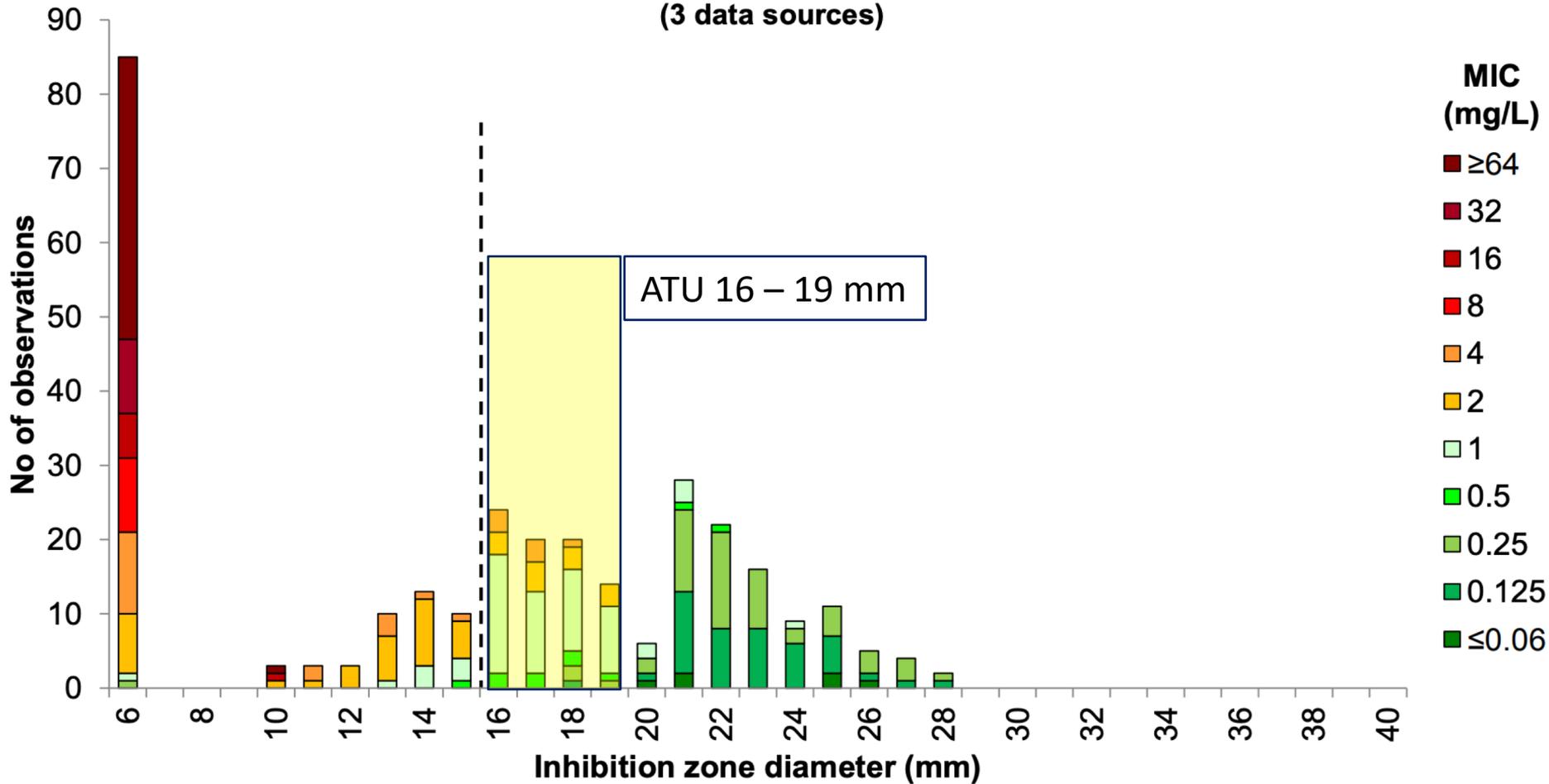
- BLA-negative
- Ampicillin zone 17 mm (S but in ATU)
- Oral step down with amoxicillin following treatment with cefotaxime (MIC 0.06 mg/L)

(do not assume the isolate is susceptible to ampicillin and amoxicillin just because it tests fully sensitive to cefotaxime)

Ampicillin 2 µg vs. MIC

H. influenzae, 166 isolates (305 correlates)

(3 data sources)



Breakpoints		ECOFF
MIC	S ≤ 1, R > 1 mg/L	1 mg/L
Zone diameter	S ≥ 16, R < 16 mm	

***H. influenzae* and PBP3 mutations**

PcG1U disk screen will always be positive

- **Child with osteomyelitis**

- BLA-negative
- Ampicillin zone 17 mm (S but in ATU)
- Oral step down with amoxicillin following treatment with cefotaxime (MIC 0.06 mg/L)

Perform amoxicillin or ampicillin BMD or choose another agent.

***H. influenzae* and PBP3 mutations**

PcG1U disk screen will always be positive

- **Case 2: a child 6 months old with otitis media.**
 - PCG-screening=R (6 mm), BLA-negative,
 - Ampicillin zone 17 mm (S but in ATU).
 - BMD is not available.
 - How to proceed....

Without access to BMD, you will need to safeguard (either report amoxicillin blank or report R) and consider a non-betalactam antibiotic.

S. aureus - ceftaroline

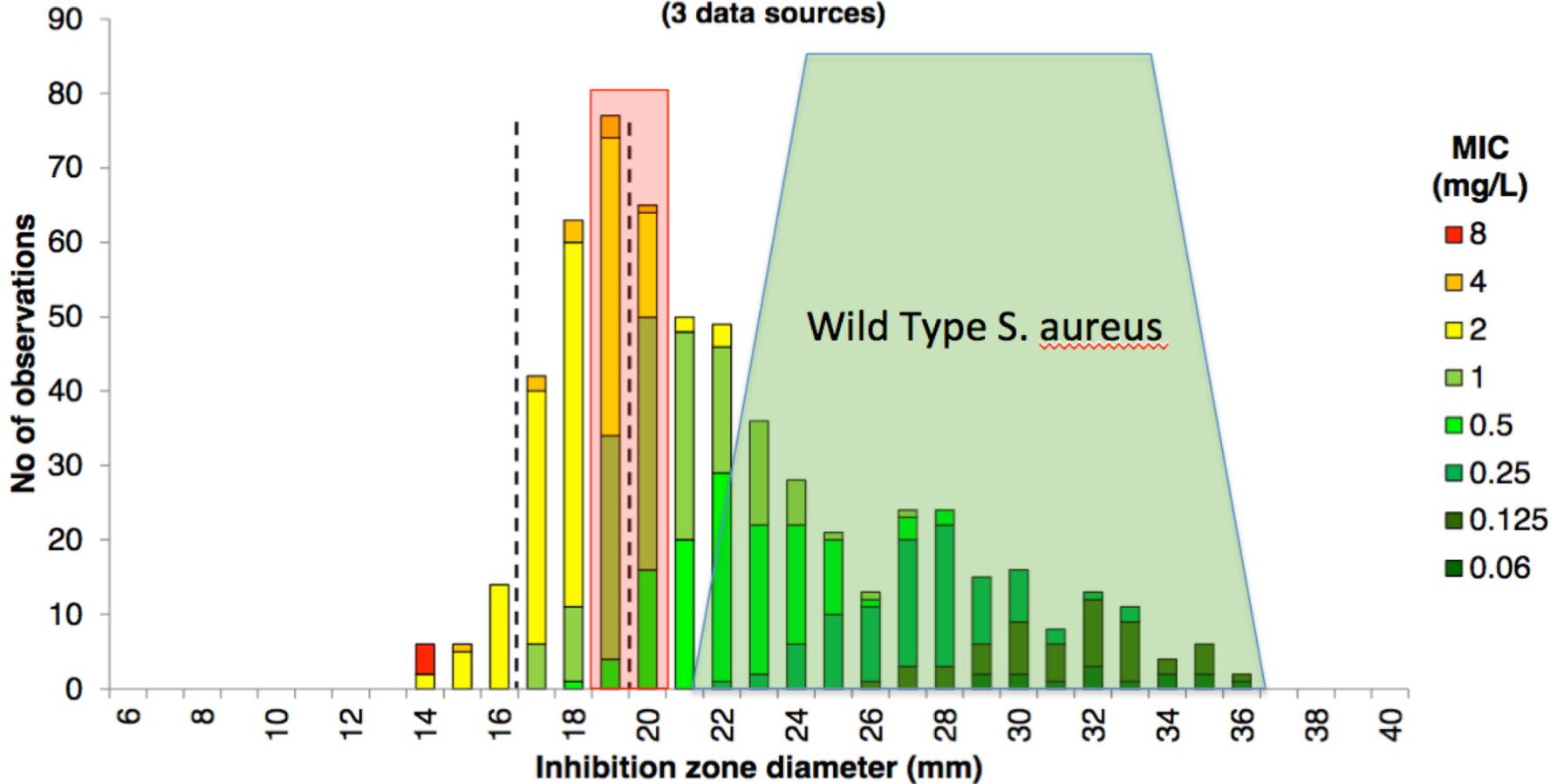
- Skin/soft tissue infection
 - MRSA, clindamycin-R
 - for various reasons, the clinician prefers to use ceftaroline* and will not consider other alternatives
 - **ceftaroline zone diameter 20 mm**, MIC not available today.

*Ceftaroline S \geq 20 R $<$ 17 mm; S \leq 1, R $>$ 2 mg/L
ATU 19 – 20 mm; MIC 1 mg/L

Ceftaroline 5 μ g vs. MIC

S. aureus, 216 isolates (593 correlates)

(3 data sources)



Breakpoints (non-pneumonia)

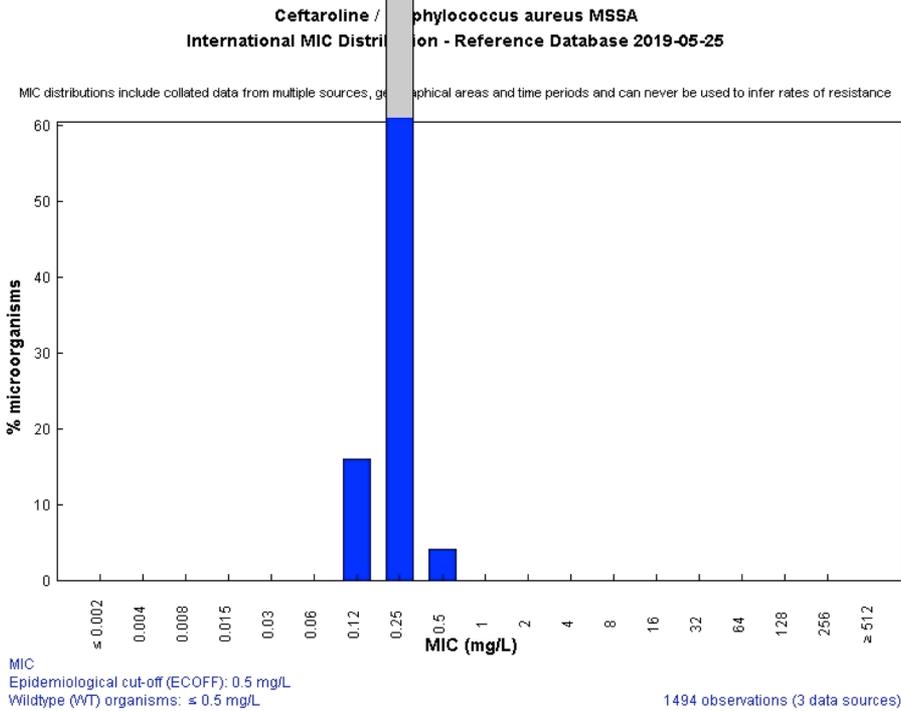
MIC $S \leq 1, R > 2$ mg/L

Zone diameter $S \geq 20, R < 17$ mm

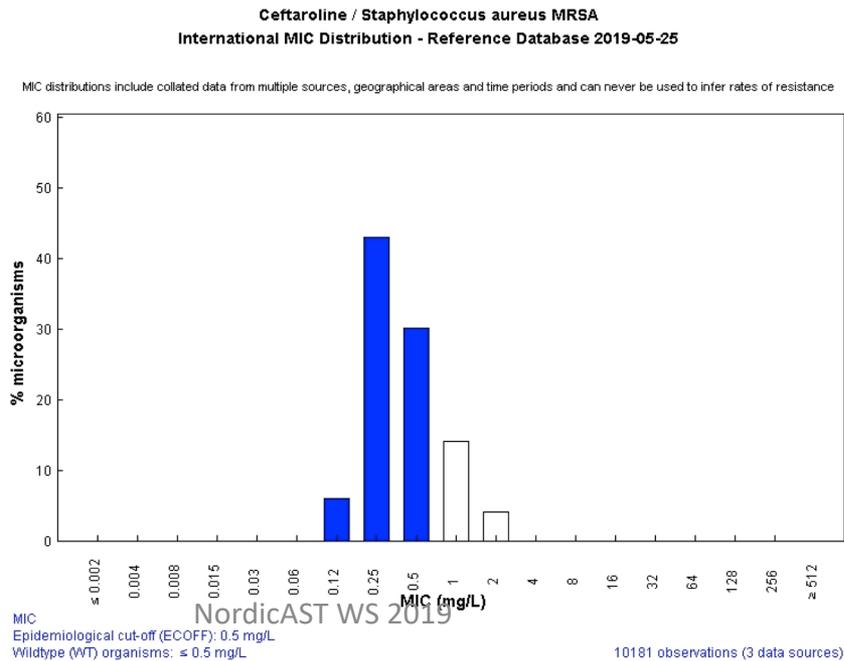
ECOFF

0.5 mg/L

In pneumonia
 $S \leq 1$, $R > 1$ mg/L
 ECOFF 0.5



Skin and soft tissue
 $S \leq 1$, $R > 2$ mg/L
 ECOFF 0.5



S. aureus - ceftaroline

- Skin/soft tissue infection
 - MRSA, clindamycin-R
 - for various reasons, the clinician prefers to use ceftaroline* and will not consider other alternatives
 - **ceftaroline zone diameter 20 mm**, MIC not available today.

*Ceftaroline S \geq 20 R $<$ 17 mm; S \leq 1, R $>$ 2 mg/L
ATU 19 – 20 mm; MIC 1 mg/L

Zone diameter=borderline S.
Wait, discuss with clinician, do not report.
Perform BMD and repeat the disk diffusion.
MIC 1mg/L, zone diameter 21 mm – **report S.**

Pseudomonas aeruginosa

- Patient with leukemia and a *Pseudomonas aeruginosa* isolate in three blood cultures, all with the same antibiogram: resistant to everything including colistin.
- Results (no other antibiotics are available for testing):
 - Piperacillin-tazobactam 6 mm,
Ceftazidime and ceftazidime-avibactam 6 mm
Ceftolozane-tazobactam 6 mm
Ciprofloxacin 6 mm, levofloxacin 6 mm
Gentamicin, tobramycin, amikacin 6 mm
Meropenem 16 mm, Imipenem 6 mm
Colistin MIC by BMD 4 mg/L.
 - Would you perform MICs for any other antibiotic (besides colistin) ?
 - How would you handle the colistin result?
 - How would you advice clinical colleagues?

The *Pseudomonas* is colistin WT.

An antibiotic with 6 mm zone will not have a useful MIC.

Perform MIC for meropenem.

Combine high dose extended infusion meropenem and colistin?

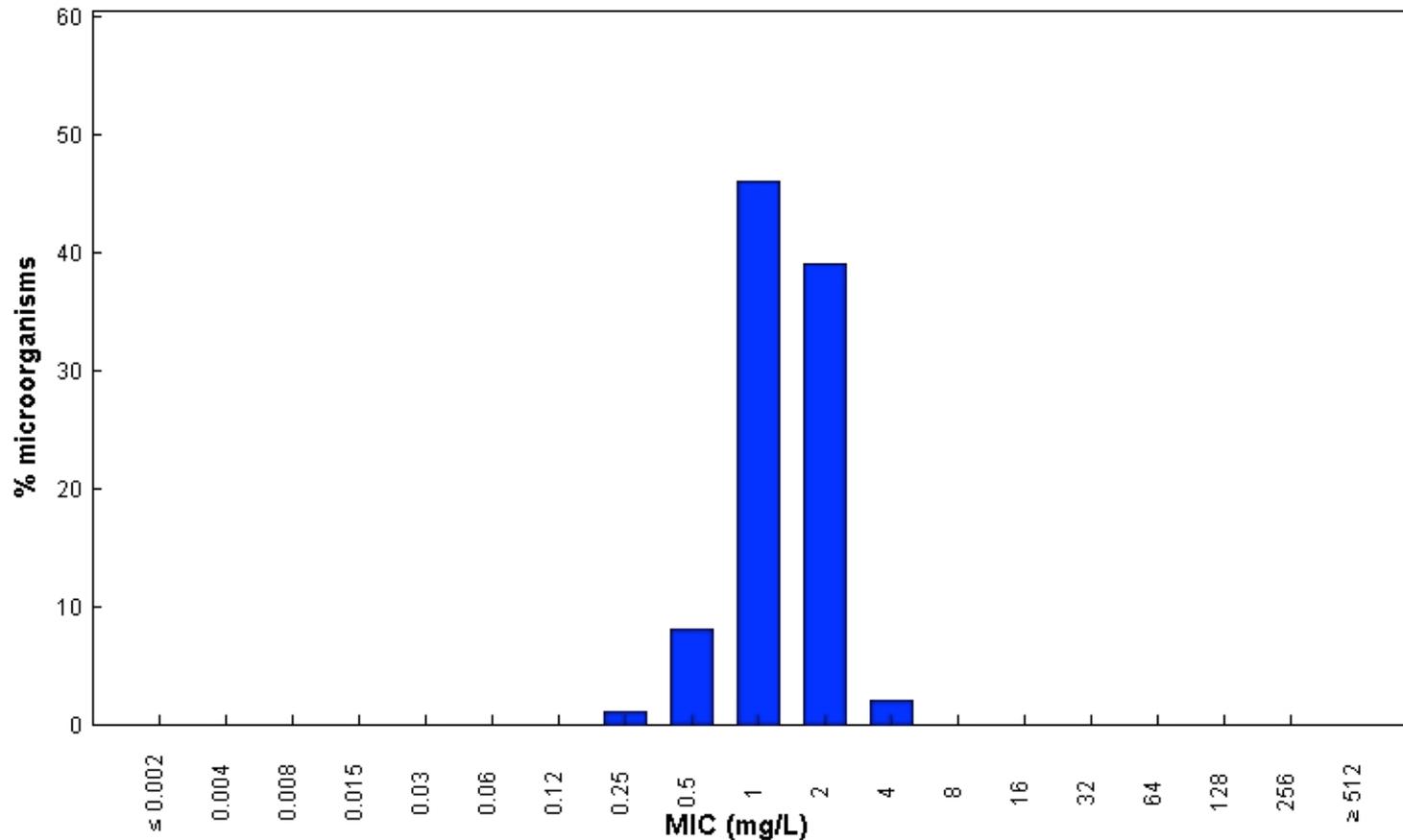
Add rifampicin (no testing)?

WGS?

Pseudomonas aeruginosa vs colistin

Colistin / *Pseudomonas aeruginosa* International MIC Distribution - Reference Database 2019-05-26

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): 4 mg/L

Wildtype (WT) organisms: ≤ 4 mg/L

Pseudomonas aeruginosa (II)

- 86-year old male with hospital-acquired pneumonia due to *P. aeruginosa*.
 - Piperacillin-tazobactam zone diameter 18 mm (ATU 17-19 mm).
 - Susceptible to ceftazidime, meropenem, ciprofloxacin, gentamicin, amikacin
 - How to proceed?

Report AST results to other antibiotics and announce that more testing on PTZ is needed OR that you will otherwise report “R”.

Perform BMD and repeat disk test!

Should we perform colistin MIC?

Current (2019) ATU warnings:

#	Species	Agent	Breakpoints ≤/>	ATU (MIC)	Breakpoints ≥/<	ATU (zone)
1	<i>Enterobacterales</i>	Amoxicillin-clavulanic	8/8	-	19/19	19-20
2	<i>Enterobacterales</i>	Piperacillin-tazobactam	8/16	16	20/17	17 - 19
3	<i>Enterobacterales</i>	Ceftaroline	0.5/0.5	-	23/23	22 - 23
4	<i>Enterobacterales</i>	Ciprofloxacin	0.25/0.5	0.5	25/22	22 - 24
5	<i>Pseudomonas</i>	Piperacillin-tazobactam	16/16	-	18/18	18 - 19
6	<i>Pseudomonas aeruginosa</i>	Ceftazidime-avibactam	8/8		17/17	16 - 17
7	<i>Pseudomonas aeruginosa</i>	Colistin	2/2	4	-	-
8	<i>S. epidermidis</i>	Cefoxitin-screen	-	-	25/25	25 - 27
9	<i>S. aureus</i>	Ceftaroline	1/1	1	20/20	19 - 20
10	<i>S. aureus</i>	Ceftobiprole	2/2	2	17/17	16 - 17
11	<i>S. aureus</i>	Amikacin	8/16	16	18/16	15 - 19
12	<i>H. influenzae</i> (PBP3 mutation)	Ampicillin	1/1	-	16/16	16 - 19
13	<i>H. influenzae</i> (PBP3 mutation)	Amoxicillin-clavulanic	2/2	-	15/15	14 - 16
14	<i>H. influenzae</i> (PBP3 mutation)	Piperacillin-tazobactam	0.25/0.25	-	27/27	24 - 27
15	<i>H. influenzae</i> (PBP3 mutation)	Several cephalosporins	See breakpoints			
16	<i>H. influenzae</i> (PBP3 mutation)	Imipenem	2/2	-	20/20	6 - 19

Questions related to the ATU

ATU for *H. influenzae* and imipenem

- ***H. influenzae*/Imipenem:**
Is the ATU really 6-19 mm?
 - **Yes, but only when the PcG1U-screen is positive.**
 - As a general rule, avoid testing imipenem for *H.influenzae*.**

**Can we adapt our breakpoints to avoid ATUs?
For example for beta-lactams and *H. influenzae***

- **No**

the problem is only with the PBP3-mutated isolates and there is no breakpoint which makes the problem disappear.

Antifungals – when will the new definition of I be implemented? ...and will there be ATUs?

- 1. No later than in breakpoint table 2020.**
- 2. Yes, most likely there will be one or two ATUs.**

ATU as a single MIC-value?

- It is fairly easy to explain to staff why there is sometimes an ATU for disk diffusion
However, I have problems explaining why some agents have an **ATU for a single MIC** value.
 - In some MIC distributions the “target +/- one dilution step” means that there is a high risk that also a single MIC-test will fail.
 - Ceftaroline and ceftobiprole are good examples of overlap
 - *Pseudomonas aeruginosa* and colistin of a breakpoint splitting the wild type population.

Our laboratory and LIS system are not ready – you are moving too fast!

- Laboratories on ADBakt/wwLab has a functioning solution in place – it is simple, tested and in operation.
- It does not interfere with your testing or reporting – **it only warns!**
- We assume you will request a “fix” from your software provider.

Antibiotikavarning (ATU)

ATU: 2

Antibiotikum: 13510 PIPERACILLIN-TAZOBAKTAM
Mikroorganism från: 23000 ESCHERICHIA COLI
till: 27290 PROVIDENCIA SPECIES

	fr.o.m	t.o.m
ATU Zon:	17	19
ATU MIC:	16	16

Kommentar: Save the isolate for further testing.
Consult the clinical microbiologist.

This is what you need in your LIS

- *S. epidermidis*
- Cefoxitin
- ATU zone: 25 – 27 mm
- ATU MIC: Not applicable

- Warning: “Before reporting the result, perform PCR to determine whether or not a *mec*-gene is present.”

If the LIS cannot handle?!

- Put up a sign with the same information
- Continue pushing the software provider to provide a solution

Thank you!



When are MICs useful?

- To confirm the uncertain result of another test.
- To systematically validate the performance of routine AST (if not MIC).
- To quantitate resistance (steer therapy) **outside** the wild type distribution.
 - There is a major difference between a meropenem MIC of 16 and 512 mg/L.
 - *S. pneumoniae* and benzylpenicillin
- To provide advice on species/agents where breakpoints are lacking.
- Slow growing organism

When are MICs **NOT** useful

- for isolates inside the wild type
 - there is no reproducible difference between organisms inside the wild type.
- if the MIC test used cannot be QC:ed because of truncated dilution series (Vitek, Phoenix etc)
- misleading when reported as precise values – MICs are best reported as a statistical range:
 - A value of 0.12 is best reported as 0.06 – 0.25 mg/L (ciprofloxacin for *E. coli* or benzylpenicillin for *S. pneumoniae*)
 - A value of 1 is best reported as 0.5 – 2 mg/L (vancomycin on *S. aureus*)